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(54) Title: HYPOCHOLESTEROLEMIC COMPOSITIONS FROM BAMBOO SHOOTS

(57) Abstract

A composition for reducing cholesterol levels in a mammal is disclosed. This composition includes a phytosterol–containing extract isolated from bamboo shoot. Pharmaceutical and dietary supplements incorporating such compositions are also provided. Methods of making and using these compositions are also disclosed.

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HYPOCHOLESTEROLEMIC COMPOSITIONS FROM BAMBOO SHOOTS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application Nos. 60/049,861 filed June 17 1997 and 60/051,818 filed July 7, 1997 which are incorporated by reference herein.

FIELD OF THE INVENTION

The present invention is a composition for lowering cholesterol levels in a mammal. More particularly, the present invention is a phytosterol-containing extract derived from bamboo shoots that lowers cholesterol by reducing or inhibiting cholesterol absorption and cholesterol synthesis and/or increasing fecal excretion of neutral and acid sterols. Methods of making and using such compositions are also provided.

BACKGROUND OF THE INVENTION

Cholesterol is associated with the pathogenesis of cardiovascular disease, which is one of the leading causes of death in the United States and developed countries. (Galton and Drone et al., 1991). Extensive epidemiological studies on the effect of cholesterol in the body have been carried out in many populations in diverse countries over the past three decades. Each study strongly suggests that a high blood cholesterol level, especially with high levels of low density lipoprotein (LDL) cholesterol, is highly associated with coronary heart disease (Goldstein and Brown, 1973).

The cause of atherosclerosis, however, by high serum total and LDL-cholesterol concentrations is not fully understood. It may involve complex detrimental interactions among lipoproteins, blood platelets, arterial endothelium, arterial smooth muscle cells, and macrophages (Ross and Golmset, 1976). These detrimental interactions are enhanced by many contributory genetic and environmental factors, including cigarette smoking, high blood pressure, and diabetes mellitus, which make certain arterial segments more susceptible than others (Sudhof et al., 1985).

One theory to explain atherosclerosis is known as the lipid oxidation theory. According to this theory, LDL particles are particularly atherogenic because either or both of their phospholipid and protein portions are chemically modified by, for example, oxidation, acetylation or glycosylation (Cathcart et al., 1986). Such modifications appear to occur largely in the walls of arteries. When these modified LDL particles are taken up by activated monocytes via scavenger receptors, these cells become laden with cholesterol (called foam cells) and develop a diminished motility. When cholesterol particles remain in the arterial wall, they form atherosclerotic plaque. High density lipoprotein (HDL) particles, however, appear either to reduce the oxidation of LDL *in vivo* or to remove the oxidized portion of the LDL particles (Parthasarathy et al., 1990).

Mammalian cells have a variety of mechanisms for regulating the metabolism of cholesterol. For example, cholesterol can be obtained from cellular metabolism via biosynthesis from acetate precursors or by absorption mediated by a LDL receptor pathway. The latter can be further subdivided into an exogenous (dietary cholesterol

absorption) pathway and an endogenous (biliary cholesterol absorption) pathway (Brown et al., 1981). Cholesterol biosynthesis and the uptake pathways are closely related and interdependent. Both pathways supply cholesterol to the body. Dietary cholesterol uptake from the gastrointestinal tract, however, can influence the rate of body cholesterol synthesis by a feed back mechanism (Dietschy et al., 1970). Overall, cholesterol from exogenous and endogenous origins are presumed to be indistinguishable and appear to have similar potential for affecting cholesterol homeostasis (Wilson and Rudel, 1994).

Three major functional tissues are involved in cholesterol metabolism: small intestine, which is the major place for exogenous and endogenous cholesterol absorption; liver, which is the dominate location for synthesizing cholesterol from acetyl-CoA; and other extrahepatic tissues mainly for cholesterol catabolism. The normal catabolic route for the disposal of cholesterol involves conversion of cholesterol into excretable bile acids in the form of neutral and acid sterols (Brown et al., 1981).

Cholesterol homeostasis is regulated and maintained by three interrelated feed back mechanisms: (1) through regulation of LDL receptor production; (2) through regulation of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase and other enzymes in the biosynthetic pathway; and (3) through regulation of cholesterol 7 alpha-hydroxylase in bile acid synthesis (Brown and Goldstein, 1986). The key regulators in these feed back mechanisms are LDL-cholesterol receptors, HMG-CoA reductase in cholesterol biosynthesis, and cholesterol 7 alpha-hydroxylase in cholesterol catabolism (Brown and Goldstein, 1986).

To combat the devastating effects of high cholesterol levels in the body, efforts have been undertaken to reduce serum cholesterol levels. Traditionally, the focus on lowering cholesterol levels has been on two approaches: (1) drug therapy and (2) dietary modification.

Drug therapies are based on the fundamental mechanism of cholesterol metabolism and homeostasis. For example, cholestyramine and colestipol are two common drugs (bile acid resins) which have been used to treat hypercholesteremia. These drugs interrupt the cholesterol enterohepatic circulation by stimulating cholesterol 7 alpha-hydroxylase activity (Galton and Krone, 1991). Blocking endogenous cholesterol synthesis in liver and peripheral tissues is another mechanism for treating hypercholesteremia. For example, lovastatin, pravastatin and simvastatin are drugs that inhibit HMG-CoA reductase synthesis by competing for receptors in the liver for endogenously produced HMG-CoA reductase (Brown and Goldstein, 1986).

The use of drugs to treat hypercholesteremia, however, suffers from several draw backs. For example, the body can become resistant to the drugs over time and may require increasingly higher doses which may cause damage to the liver or other organ systems. Such drugs may also cause undesirable side effects in patients and must be closely monitored by a physician. Moreover, the drugs are expensive. Accordingly, the use of these drugs is always a last resort. Thus, attempts have been made to design low cholesterol diets to lower cholesterol levels in patients without having to resort to the more extreme measures of conventional drug therapy as set forth above.

For example, studies over the last four decades indicate that the effect of diet on the risk of coronary heart disease is strong. In these studies, many dietary or food sources have been extensively studied. These food sources include different types of fat, complex and simple carbohydrates, animal and plant proteins, vitamins and minerals and different types of dietary fibers.

Traditionally, studies of the effect of diet on serum cholesterol have focused on medium-chain fatty acids, soluble fiber and dietary cholesterol. In recent years, great effort has been expended to identify new cholesterol-lowering substances in fruits and vegetables. Such fruits and vegetables contain many unique and complex organic compounds, some of which are biologically active. In particular, certain phytochemicals have been identified from fruits and vegetables which are biologically significant. An example of such a phytochemical is a family of plant or vegetable sterols known as phytosterols which are generally classified into three groups: 4-desmethylsterols, 4-monomethylsterols and 4,4'-dimethylsterols. It is known that certain of these phytosterols have a therapeutic effect on lipid metabolism.

Moreover, it is known that certain fruits and vegetables produce hypocholesterolemic effects when consumed as part of a diet. Little research has been published, however, on the physiologic effects produced by such fruits and vegetables. In one study it was suggested that consumption of bamboo shoots in a diet reduced weight and decreased serum cholesterol in rats (Chang 1993). In this study, the author attributed these effects to the high fiber content of bamboo shoots.

The present inventors, however, have demonstrated surprisingly that the cholesterol lowering effect observed by Chang in 1993 is not caused by the fiber content of the bamboo shoots. Rather, the inventors have discovered that the cholesterol lowering effect is caused by phytosterols present in bamboo shoot.

Thus, it is an object of the present invention to provide a composition for lowering cholesterol levels in a mammal that includes a phytosterol-containing extract isolated from bamboo shoot. It is a further object of the present invention to provide pharmaceutical compositions and dietary supplements that include phytosterol-containing extracts isolated from bamboo shoots that are effective for lowering cholesterol levels in a mammal. It is another object of the present invention to provide methods of making and using such compositions for lowering cholesterol levels in a mammal. The present invention is directed to meeting these and other needs.

SUMMARY OF THE INVENTION

The present invention is a composition for reducing cholesterol levels in a mammal. This composition includes a phytosterol-containing extract isolated from bamboo shoot.

Another embodiment of the present invention is a dietary supplement that includes as an active agent a cholesterol lowering amount of a phytosterol-containing extract isolated from bamboo shoot.

A further embodiment of the invention is a pharmaceutically useful composition that includes an extract containing one or more phytosterols isolated from bamboo shoots.

A further embodiment of the invention is a method for lowering cholesterol levels in a mammal. This method includes administering to a mammal a composition that includes an effective amount of a phytosterol-containing extract isolated from bamboo shoots sufficient to lower cholesterol levels in the mammal.

The present invention also includes a method of making a composition for lowering cholesterol levels in a mammal. This method includes obtaining an extract of phytosterols from a source of bamboo shoots and combining the extract with a suitable delivery vehicle for administering cholesterol-lowering amounts of the extract to the mammal.

Another embodiment of the present invention is a method for inhibiting cholesterol absorption and/or increasing fecal excretion of neutral and acid steroids in a mammal. This method includes administering to the mammal an effective amount of a composition having as its primary active agent one or more phytosterols isolated as an extract from bamboo shoots.

A further embodiment of the present invention is a method of inhibiting cholesterol synthesis by administering to a mammal a cholesterol inhibiting amount of a composition. This composition includes an extract containing one or more phytosterols isolated from bamboo shoot.

Another embodiment of the present invention is a method for reducing cholesterol levels in a mammal. This method includes in combination inhibiting cholesterol absorption and inhibiting cholesterol synthesis by administering to the mammal an effective amount of a composition that includes an extract of one or more phytosterols isolated from bamboo shoot.

Yet another embodiment of the present invention is a method for lowering cholesterol levels in a mammal by reducing or inhibiting cholesterol synthesis and cholesterol absorption. This method is achieved by administering to the mammal cholesterol lowering amounts of bamboo shoots.

BRIEF DESCRIPTION OF THE DRAWINGS

For a fuller understanding of the invention, reference is had to the following description taken in connection with the accompanying drawings in which:

FIG. 1 is an outline of the cholesterol biosynthesis pathway.

FIG. 2 is an outline of the pathway by which cholesterol is converted into the primary bile acids.

FIG. 3 is a table showing the formulation of two embodiments of the present invention and two control formulations.

FIG. 4 is a table showing the cholesterol-lowering effects of two embodiments of the present invention vs. control formulations in a hypercholesterolemic model in rat.

FIG. 5 is a table showing the effects on rat liver weights and liver lipid profile of the formulations of FIG. 3.

FIG. 6 is graph of total cholesterol levels measured in rats comparing the formulations of FIG. 3.

FIG. 7 is a table showing TDF, SDF, IDF and certain phytosterols contained in the formulations of FIG. 3.

FIG. 8 is a table showing the effects of the formulations of FIG. 3 on fecal bile acid steroids, neutral steroids and total steroids in rat.

FIG. 9 is a table showing the effects of the formulations of FIG. 3 on output of certain fecal neutral phytosterols in rat.

FIG. 10 is a table showing the effects of the formulations of FIG. 3 on output of certain neutral phytosterols in rat.

FIG 11 is a table showing the effects of the formulations of FIG. 3 on output of certain fecal acid steroids in rat.

FIG. 12 is a table showing the effects of the formulations of FIG. 3 on certain fecal bile ratios in rat.

FIG. 13 is a process flow chart for the extraction and fractionation of compositions of the present invention from bamboo shoot by liquid and lipid extraction.

FIG. 14 is a preparative reverse phase HPLC chromatogram of a crude bamboo shoot methanol soluble fraction of FIG. 12.

FIG. 15 is a preparative normal phase HPLC chromatogram of a methanol insoluble bamboo shoot fraction of FIG. 12.

FIG. 16 is a graph showing a dose response curve of the crude bamboo shoot extract of FIG. 12 on Hep G2 cell cholesterol content.

FIG. 17 is a graph showing a time course study on the effect of the crude bamboo shoot extract of FIG. 12 on Hep G2 cell cholesterol content.

FIG. 18 is a graph showing the effect of various fractions of FIG. 12 on Hep G2 cell cholesterol content.

FIG. 19 is an HPLC chromatogram of the methanol soluble fraction of FIG. 12.

FIG. 20 is a gas chromatogram of the total crude extract of FIG. 12.

FIG. 21 is a gas chromatogram of the total methanol insoluble fraction of the total crude extract of FIG. 12.

FIG. 22 is a table listing the phytosterols identified in the chromatogram of FIG 21.

FIG. 23 is a table listing major peak mass spectra of the phytosterols identified in the chromatogram of FIG. 21.

FIG. 24 is an electron ionization mass spectrum of the F1 fraction of the total methanol soluble fraction of the total crude extract of FIG. 12.

FIG. 25 is the electron ionization mass spectrum of the F5 fraction of the total methanol soluble fraction of the extract of FIG. 12.

FIG. 26 is a graph showing the effect of various fractions of the extract of FIG. 12 on the expression activity of HMG-CoA reductase in Hep G2 cells.

DETAILED DESCRIPTION OF THE INVENTION

The present invention includes a composition for reducing cholesterol levels in a mammal. This composition includes a phytosterol-containing extract isolated from bamboo shoot. As used herein, the term "phytosterol" includes the entire group of free

phytosterols, phytosterol fatty acid esters and (acylated) phytosterol glucosides. The present invention is based in part on the Ph.D. thesis of one of the applicants entitled "The Hypocholesterolemic Effect of Bamboo Shoot *In Vivo* and *In Vitro*" submitted to The Graduate School, New Brunswick of Rutgers, The State University of New Jersey the entire contents of which is incorporated by reference.

As used herein, "reducing cholesterol levels" means that the present compositions when administered to a mammal are able to reduce serum total cholesterol, LDL cholesterol and total liver lipids. For purposes of the present invention, "total liver lipids" includes liver cholesterol, as well as liver triglycerides.

As set forth in more detail in the examples *infra*, the active agent or agents in the present compositions are derived from a crude extract of bamboo shoots. The crude extract has been fractionated and analyzed for the presence of phytosterols. In particular, several phytosterols have been identified in various fractions of the crude extract using conventional analytical techniques, such as for example, gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS). As set forth in more detail in the examples, each fraction of the crude extract contains one or more phytosterols.

Useful extracts of the present compositions contain a mixture of phytosterols therein including, for example, sitosterol, sitastanol, stigmasterol and derivatives and isomers thereof. The extracts of the present invention can also include, for example, beta-

sitosterol, stigmasta-3,5-dien-7 one, stigmast-4-en-3-one, stigmasta-5,22-dien-3-ol, campesterol, and derivatives and isomers thereof. For purposes of the present invention, the term "derivatives" is intended to encompass all chemically modified versions of the enumerated phytosterols which alone or in combination have a cholesterol lowering effect when administered to a mammal. For example, chemically modified forms of phytosterols that are useful in the present invention include esterified, glycosidic, saturated or unsaturated and oxysterol forms thereof.

The term "bamboo" or "bamboo shoot" is used throughout the specification. For purposes of the present invention, "bamboo" or "bamboo shoot" means species of bamboo that belong to the family *Gramineae* (Yamaguchi, 1983), which is a perennial grass. Bamboos have great variation in clump height and diameter. Bamboos are divided into two classes on the basis of their vegetative growth habits: clump-forming types and spreading types. The clump forming types of bamboo produce underground stems called "rhizomes", which grow horizontally only a few inches from the base of the existing clump and turn up to form very compact clumps. The spreading type of bamboo grow horizontally several feet and form open clumps. The clump-forming bamboos generally are tropical types, whereas, the spreading bamboos usually grow in temperate regions of the world (Kennard and Freyne, 1957).

Bamboo shoots are underground sprouts of bamboo. Bamboo shoots are divided into two categories, one comes from tropical clump bamboos, such as *Bambusa oldhami Nakai* and *Dendrocalamus latiflorus* Munro; the other comes from temperate spreading

bamboos, such as *Phyllostachys edulis*, *P. pubescens*, and *P. makinoi* (Hui, 1992).

The present compositions include phytosterols derived from bamboo shoot obtained from a variety of bamboo species including, for example, *Bambusa oldhami Nakai, Bambusa edulis, Pseudosas usawai, Zizania latiflia, Saccharum officinarum, Dendrocalamus latiflorus* Munro, *Phyllostachys edulis, Phyllostachys pubescens*, and *Phyllostachys makinoi*.

In the present invention, pharmaceutical compositions which lower cholesterol levels in mammals can be formed from phytosterols extracted from bamboo shoot. These compositions include a therapeutically effective amount of the crude phytosterol extract and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. The exact formulation, of course, will suit the mode of administration.

The pharmaceutical compositions of the present invention, if desired, can also contain minor amounts of wetting or emulsifying agents or pH buffering agents. These compositions can take various forms including, for example, solutions, suspensions, emulsions, tablets, pills, capsules, sustained release formulations or powders. These compositions can be formulated as a suppository with traditional binders and carriers, such as triglycerides. Oral formulations are also contemplated and can include standard carriers, such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc.

These compositions can be formulated for intravenous administration to mammals. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffers. Where necessary, these pharmaceutical compositions may also include a solubilizing agent and a local anesthetic, such as lidocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container, such as an ampule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampule of sterile water or saline for injection can be provided so that the ingredients may be mixed prior to administration.

The compositions of the present invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups, such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc. and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of phytosterol extract which will be effective in the treatment of a particular disorder or condition, such as for example, hypercholesteremia, will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. As used herein, "disorder" or "condition" can include, for example

hypercholesteremia, cancer, in particular colon cancer, benign prostatic hyperplasia, atherosclerosis caused by platelet aggregation and/or smooth muscle cell proliferation and inflammation.

The precise dose to be employed in the formulation will also depend on the route of administration and should be decided according to the judgment of a physician and each patient's circumstances. Suitable dosage ranges for intravenous administration, however, are generally about 20-500 micrograms of active compound per kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body weight. Effective doses may be extrapolated from does response curves derived from *in vitro* or animal model test systems.

Suppositories generally contain active ingredient in the range of 0.5% to 10% by weight; oral formulations preferably contain 10% to 95% active ingredient.

The present compositions can also be incorporated into dietary supplements. Such supplements include as an active ingredient effective amounts of the present composition to lower cholesterol levels in a mammal. The formulation of dietary supplements is well known in the art and can include a suitable carrier, as well as minor amounts of a variety of materials including for example, wetting or emulsifying agents or pH buffering agents.

The specific formulation of the dietary supplements of the present invention will vary depending upon a number of factors, including the sex and weight of the patient, as

well as the severity of the disease. The dietary supplements of the present invention, however, must include a sufficient amount of crude phytosterol extracts from bamboo shoot to lower cholesterol levels in a patient. In particular, the extract in the supplement must lower serum total cholesterol, LDL cholesterol, as well as total liver lipids, including for example, liver cholesterol and liver triglycerides.

As set forth previously, the dietary supplement includes a crude extract that contains one or more phytosterols derived from bamboo shoots. These extracts can include, for example, sitosterol, sitastanol, stigmasterol and derivatives and isomers thereof. The present extracts can also include, for example, a mixture of phytosterols including beta-sitosterol, stigmasta-3,5-dien-7 one, stigmast-4-en-3-one, stigmasta-5,22-dien-3-ol, campesterol, and derivatives and isomers thereof. For purposes of the present invention, the term "derivatives" is intended to include chemically modified phytosterols that maintain their ability to lower cholesterol in a mammal. Such derivatives include for example, esterified, glycosidic, saturated or unsaturated and oxysterol forms of the phytosterols found in the bamboo shoot extracts.

The present invention also includes a method for lowering cholesterol levels in a mammal by administering to the mammal an effective amount of a phytosterol-containing extract isolated from bamboo shoots sufficient to lower cholesterol levels. As used herein, the term "mammal" includes humans, as well as other species.

Another embodiment of the present invention includes a method of making the

compositions for lowering cholesterol levels in mammals as set forth above. This method includes obtaining an extract from a source of bamboo shoots that contains a mixture of phytosterols and combining that extract with a suitable delivery vehicle for administering cholesterol-lowering amounts of the extract to a mammal. The delivery vehicle can be any physiologically appropriate carrier for administering the cholesterol lowering extracts of the present invention to a mammal. These delivery vehicles have been described in detail above and include both pharmaceutical preparations, as well as dietary supplements.

The mechanism by which the present compositions act *in vivo* to lower cholesterol levels is poorly understood. Not wishing to be bound by any particular theory, Applicants believe that the present compositions lower cholesterol levels in mammals through inhibition of cholesterol absorption and/or modification of bile acid excretion. Another possible mechanism involves the lipid metabolic and cholesterol synthetic pathways.

As set forth in more detail in the examples, Applicants have conducted *in vivo* studies in a rat model of hypercholesteremia using preparations containing the presently described phytosterol compositions at two different concentrations (15% and 30%, respectively). These results indicate that the administration of bamboo shoot or extracts thereof to hypercholesteremic rats significantly increase fecal cholesterol, coprostanol, cholic acid and phytosterol output as compared to controls. In this model, a majority of the bamboo shoot phytosterols appear to be absorbed while the rest were recovered in the feces. As the results demonstrate, the lower concentration preparation (15% bamboo shoot) significantly increased fecal chenodeoxycholic acid output indicating that the

present compositions inhibit cholesterol absorption. The higher concentration preparation (30% bamboo shoot) significantly increased cholic acid output indicating that the present compositions also decrease cholesterol absorption while decreasing hepatic cholesterol synthesis.

These results further indicate that phytosterols in bamboo shoot play a significant role in reducing cholesterol levels in mammalian models, whereas dietary fiber plays a relatively minor role. To the best of Applicants' knowledge, the data set forth herein represent the first *in vivo* and *in vitro* demonstration of the cholesterol lowering effect obtained with phytosterols derived from bamboo shoot. These data also show that the present compositions significantly decrease the ratio of secondary bile acids to total bile acids and the ratio of chenodeoxycholic acid to cholic acid. Accordingly, these data indicate that the present compositions also play a key role in the health of the colon.

Accordingly, another embodiment of the present invention includes a method of inhibiting cholesterol absorption and/or increasing fecal excretion of neutral and acid steroids in a mammal. This method includes administering to a mammal an effective amount of a composition having as its primary active agent one or more phytosterols isolated as an extract from bamboo shoot as set forth previously.

In this method, the compositions of the present invention can be in any form conveniently administered to a mammal. For example, the compositions can be administered as a pharmaceutical preparation, a dietary supplement or as fresh or

desiccated bamboo shoot consumed alone or in combination with food or drink. In mammals, compositions administered according to this method decrease serum total and LDL cholesterol, as well as total liver lipids, including for example, liver cholesterol and liver triglycerides.

Another embodiment of the present invention includes inhibiting cholesterol synthesis by administering to a mammal a cholesterol inhibiting amount of a composition according to the present invention. As set forth previously, this composition includes an extract containing one or more phytosterols isolated from bamboo shoot.

To further elucidate the biochemistry of the present compositions, bamboo shoot was fractionated by column chromatography. When total crude bamboo shoot extract isolated using column chromatography was applied to human hepatoma cells (Hep G2), mRNA expression of HMG-CoA reductase was significantly decreased. HMG-CoA reductase is the rate limiting enzyme in the cholesterol biosynthetic pathway which converts acetyl CoA to mevalonate and further to cholesterol or to a number of non-sterol isoprenoids (FIG. 1). Thus, it appears that the phytosterols present in such extracts are able to prevent one or more enzymes in the cholesterol biosynthetic pathway from effectively synthesizing cholesterol *in vivo*.

Surprisingly, it was found that only total crude bamboo shoot extract significantly down regulated HMG-CoA reductase mRNA transcription in the Hep G2 cells. Moreover, using liquid chromatography techniques including high pressure liquid chromatography

(HPLC), as well as mass spectrometry, 17 phytosterols were identified in the chromatographed bamboo shoot samples. Not wishing to be bound by any particular theory, it is believed that the presence of multiple phytosterols in the crude extract may explain why the crude extract alone showed significant HMG-CoA reductase mRNA synthesis down-regulation. In particular, the presence of multiple phytosterols in the crude extract may function synergistically or act at multiple sites in the cholesterol biosynthetic pathway to account for the HMG-CoA reductase mRNA synthesis down-regulation.

Among the 17 phytosterols observed by the inventors, 5 have been identified as stigmasta-3,5-dien-7one, stigmast-4-en-3-one, beta-sitosterol, stigmasta-5,22-dien-3-ol and campestrol. The phytosterols in the crude extract are structurally diverse. For example, beta-sitosterol and campesterol are common sterols in plant leaves. Stigmasta-3,5-dien-7-one and stigmast-4-en-3-one are sterol derivative products which are post mevalonate metabolites. Furthermore, two saturated phytosterols were found in the crude extract which are believed to be isomers of sitostanol. To the best of Applicants' knowledge, the observation of the presence of 17 phytosterols in bamboo shoot has never been reported.

Thus, a further embodiment of the present invention includes a method for reducing cholesterol levels in a mammal. This method includes in combination, inhibiting cholesterol absorption and inhibiting cholesterol synthesis by administering to the mammal an effective amount of a composition according to the present invention. As set forth above, this composition includes an extract of one or more phytosterols isolated from bamboo shoot.

In this method, cholesterol synthesis is inhibited by suppressing or decreasing expression of one or more enzymes in the cholesterol biosynthesis pathway, such as, for example by inhibiting HMG-CoA reductase mRNA activity. The present compositions can be administered to a mammal in any convenient form, such as for example, as a pharmaceutical or a dietary supplement together with any physiologically suitable carrier.

A further embodiment of the present invention is a method for lowering cholesterol levels in a mammal by reducing or inhibiting cholesterol synthesis and cholesterol absorption. This method includes administering to a mammal a cholesterol lowering amount of bamboo shoots. In accordance with this method, the bamboo shoots are ground into a powder using any conventional technique. The powder is then combined with a food source, a dietary supplement or a pharmaceutical composition. In the present method, the bamboo shoots are administered to a mammal in any convenient form, such as for example, as a fresh food source or as a dried source.

The following examples are provided to further illustrate the compositions and methods of using and preparing the present phytosterol-containing compositions, as well as certain physical properties thereof. These examples are illustrative only and are not intended to limit the scope of the invention in any way.

EXAMPLE 1
Hypocholesterolemic Effect of Bamboo Shoot on Serum and Hepatic Lipids in the Rat

The objective of this experiment was to investigate the effects of dietary bamboo shoot on lipid metabolism in the rat through the determination of plasma cholesterol and liver lipids under hypercholesterolemic diet conditions.

Animals

Male adult Wistar rats (Charles River Laboratory, Wilmington, MA) weighing about 200 grams were housed individually in stainless steel wire cages in a controlled environment with 12-hr light and dark cycles plus low background noise and controlled temperature (22±1°C). The duration of the study was for four weeks. Rats were fed *ad libitum*, and had free access to tap water. A one week adjustment period preceded the experimental phase. The rats were assigned to four dietary treatment groups (7 rats per treatment) by selective randomization (blocked by weight, one rat per treatment group from each block).

All rats were fed a semipurified diet and each treatment was controlled by adding a different source of "fiber" (FIG. 3). The four treatment groups consisted of (1) a wheat bran diet (containing 15% wheat bran); (2) an oat bran diet (containing 15% oat bran); (3) a bamboo shoot diet I (containing 15% by weight bamboo shoots); and a bamboo shoot diet II (containing 30% bamboo shoots by weight). For this experiment diets (1) and (2) above where controls and (3) and (4) represented formulations according to the present invention.

Diets

Wheat bran and oat bran were kindly provided as gifts by the Lauhoff Grain Co. (Illinois) and the Quaker Oats Company (Illinois), respectively. Canned winter bamboo shoots (*Phyllostachys edulis*) used in the study were processed by the Meiling Co. (China) and purchased from a local supermarket. Water was drained from the canned winter bamboo shoots. The shoots were then sliced and freeze-dried in the Food Science Pilot Plant at Rutgers University. The dried shoots were ground through a 1 mm sieve Fitz mill to a fine powder, sealed in glass jars and stored at -20°C for subsequent use.

The formulations of the four diets in FIG. 3 were based on the AIN 76A semi-purified rodent diet. The experimental design is also based on a proximate analysis of the three fiber sources, i.e., wheat brand, oat brand and bamboo shoot. These four diets were blended and pelleted by Research Diets Inc., New Brunswick, NJ. The basic diet provided a sufficient balance of nutrients for rats to maintain body weight.

Bile salt and cholesterol are necessary to enhance hypercholesterolemia in the rodent model (Shinnick et al., 1988; Story et al., 1974). Thus, cholesterol (1%) and sodium cholate (0.1%) were added to each formulation to make the diet hypercholesterolemic and to elevate liver cholesterol concentrations. In some instances, cholesterol was fed to the rats together with bile acids or bile salts.

Animals in each of the four treatment groups were provided with new food every two days. Body weight and food intake were recorded three times a week. At the end of

each experimental week, animals were deprived of feed for 16 hours and then blood was drawn.

In particular, 1 ml of blood was drawn through the rat tail vein of each animal. After allowing the blood to stand and clot for 30 min, it was centrifuged at 2,500 x g for 25 min. at 4 °C to obtain serum. Serum was immediately frozen at -70 ℃ for future analysis. At day 30, animals were weighed, blood was collected and serum was prepared. All animals were then scarified for tissue collection. Livers were excised, rinsed, blotted, weighed, and stored in dry ice. The liver samples were then divided into several one gram samples and stored at - 70 °C for future analyses. Other tissues, such as kidney, heart, spleen, adipose pad were also excised, rinsed, blotted, weighed and recorded.

Total serum cholesterol, high density lipoprotein (HDL) cholesterol, and triglycerides were determined by enzymatic colorimetric methods for cholesterol, HDL and triglycerides (diagnostic kit 0599, 0598, 2000, Stanbio Laboratory, Inc., San Antonio, Texas). The value of low density lipoprotein (LDL) cholesterol was calculated by the formula: (total Chol.) - (HDL) - (Triglycerides/5). Liver total lipid was determined by extraction of total lipids by the gravimetric method (Folch et al., 1957). Liver cholesterol was determined in aliquots (30 µl) of extract after evaporation under nitrogen and solubilizing with Triton X-100 (Carlson and Goldfarb 1977), using the same enzymatic kit (# 0059) as for serum. Total liver triglycerides were determined as described by Fletcher (1968). Data were statistically analyzed using Analysis of Variance and Scheffe's test by using the statistic analysis system (SAS). Differences are considered significant at $P<0.05$.

We examined the effects of the four experimental diets on organ weights, such as heart, kidney, adipose pad, lung and spleen (FIG. 5). As documented in FIG. 5, only bamboo shoot diet II (30% bamboo shoot) significantly lowered liver weight. No other organ weight, however, was affected by the dietary treatment. Accordingly, these data together with the data set forth in FIGS. 4 and 7 indicate that high dosages of bamboo shoots are able to prevent liver lipid accumulation because of a reduction in liver triglyceride and cholesterol.

Serum lipid profiles as a result of the four experimental diets are shown in FIG. 5. Serum total cholesterol levels in both bamboo shoot treatment groups I and II (82 mg/dl for bamboo shoot I diet, 64.5 mg/dl for bamboo shoot II diet) were significantly lower than that of the two control treatments (128.7 mg/dl on the wheat bran diet and 117.9 mg/dl on the oat bran diet). The two different bamboo shoot diets had significantly different impact on total serum cholesterol levels. The high dosage bamboo shoot diet (30% bamboo shoot) induced a higher (about 50%) reduction in total serum cholesterol as compared to the value for the control fiber diets. Whereas, the lower bamboo shoot diet resulted in a reduction (about 30%) in comparison to the fiber control diets.

The pattern of change in LDL-cholesterol in the four dietary treatments mimic those of total cholesterol. LDL-cholesterol levels in bamboo shoot diet I (42.5 mg/dl) and in bamboo shoot diet II (25.5 mg/dl) were significantly lower than those of the two control treatments groups (89.2 mg/dl in wheat bran diet and 82.4 mg/dl in oat bran diet.) (P=0.001). The higher dose (30%) bamboo shoot diet II lowered the LDL-cholesterol by

about 60% and the lower dosage (15%) bamboo shoot diet I lowered the LDL-cholesterol by 50%.

Serum HDL's were shown to be significantly different among the four treatments (P=0.02). Both bamboo shoot treatment groups exhibited a slight but significantly higher HDL level as compared with the oat bran control groups.

The HDLC/LDLC ratios in both bamboo shoot treatment groups (0.66 in bamboo shoot diet I and 1.21 in bamboo shoot diet II) were significantly higher than those of oat bran (0.29) and wheat bran (0.30). Since the HDL to LDL ratio is a clinical index of heart protection, the significantly higher ratio of HDL to LDL in both bamboo shoot treatments indicates that bamboo shoot is protective and the response is dose related. Triglyceride values in the four treatments were not significantly different from each others (P=0.07).

2) Liver Lipids

Liver weight and lipid content per 100 g of fasting body weights are shown in FIG. 5. Rats fed the high dosage bamboo shoot diet (30%) had significantly lower liver weights per 100 g of body weight compared to those of the other three treatment groups. Lower liver weights were not attributable to lower feed intake or feed efficiency. The mean liver weight in the bamboo shoot I diet was also lower than that of the other two control treatments, but the value was not statistically significant.

Total liver total lipid per 100g of liver weight (17.8 g) was significantly lower in the rats fed the bamboo shoot diet II. It appears that whereas the triglyceride component of the total liver lipid does not change with high dosage bamboo shoot diet II, the cholesterol levels are significantly changed. These data indicate that bamboo shoot, in a dose-dependent manner, played an important role in reducing liver lipid content and preventing cholesterol infiltration in the liver of cholesterol-fed rats.

3) Liver Triglycerides and Liver Cholesterol

Liver triglycerides were significantly lower in rats fed the bamboo shoot diet II (43.6 mg/g) compared to controls. The triglyceride value for bamboo shoot diet I (63.4 mg/g) was not significantly different from the two control diets. Additional bamboo shoot (increased dose) appears to lower liver triglycerides, as well as liver weight.

There were significant decreases in liver cholesterol in the bamboo shoot diet I (46 mg/g), and bamboo shoot diet II (24 mg/g) as compared to the control diets. This result is consistent with earlier observations that the presence of dietary bamboo shoots significantly decrease liver cholesterol content compared to control diets.

4) Total Serum Cholesterol Levels

Serum cholesterol concentrations changed over time in the four experimental groups (FIG. 6). When 1% cholesterol and 0.1% sodium cholate were added to the wheat bran and oat bran diets, elevated serum cholesterol levels were observed, but bamboo shoot

diets I and II inhibited this elevation.

As shown in FIG. 6, the effect of bamboo shoot diet I (BS I) on serum cholesterol was flat at 80 mg/dl, indicating that this amount of bamboo shoot suppressed cholesterol elevation expected with a hypercholesterolemic diet. Higher doses of bamboo shoots (BS II - 30% bamboo shoot) overcame the cholesterol and cholate addition and serum cholesterol decreased.

EXAMPLE 2
Effect of Dietary Bamboo Shoot on Fecal Steroid Excretion in the Rat

Adult male Wistar rats were obtained, divided into 4 experimental groups and fed 4 different diets as set forth in FIG. 3 and described in detail in Example 1.

Preparation of Fecal Sample Neutral and Acid Sterols

Rat feces were collected for 7 consecutive days of the last week of the experiment and stored at -20°C for further analysis. For sterol and bile acid extraction, fecal samples from individual rats were weighed and dried, and ground in a homogenizer. Neutral and acidic sterols were determined according to the methods of Grundy (1965), Miettinen and Ahrens (1965), Miettinen et al., (1982) and Czubayko et al., (1991). Briefly, fecal samples were thawed overnight and homogenized with distilled water (1:1, w/w). 1 mg 5 alpha-cholestane (Fisher Scientific Co., Pittsburgh, PA) and 1 mg 23-nordeoxycholic acid (Fisher Scientific Co., Pittsburgh, PA) were added to the feces samples as internal standards for

neutral and acidic sterols, respectively. A 1 gram sample was hydrolyzed in mild alkali (10 ml 1 N NaOH in 90% ethanol) for 1 hour in a water bath at 67°C. After the sample was cooled to room temperature, 5 ml of water was added and the neutral sterols were extracted 3 times with 10 ml cyclohexane. The lower aqueous phase was stored for acidic sterol analysis. Cyclohexane phases were combined and evaporated to dryness under a stream of dry N_2. 1.5 ml of TMS-reagent (dry pyridine-hexamethyldisilazane-trichlorosilane, 9:3:1 (Supelco, Bellefonte, PA)] was added to the sample in order to convert it to trimethylsilyl (TMS)-ether. After 30 min. at room temperature, the mixture was evaporated to dryness under dry N_2. 2 ml of n-decane was used to dissolve TMS-derivatives, followed by a 10-min centrifugation at 2000 U/min. A 1 ml sample was transferred to a glass vial for subsequent gas-liquid chromatographic (GLC) analysis.

2 ml 10 N NaOH was added to the lower aqueous phase. After heating for 3 h at 120°C, 5 ml H_2O was added to the mixture. After cooling to room temperature, the samples were acidified to pH<1.5 with 25% HCl. The acidic sterols were extracted three times with 10 ml diethyl ether. The combined ether phases were evaporated to dryness under N_2. For methylation, 2 ml dried methanol, 1.4 ml dimethoxypropane, and 20 ul concentrated HCl were added to the sample. The sample was mixed thoroughly and allowed to stand at room temperature for at least 1 h. After evaporation to dryness, bile acids were derivatized to their respective TMS-ethers as described for neutral sterols. 2 ml of n-decane was added to the sample, centrifuged and a 1.0 ml aliquot was transferred to glass vials for GLC analysis.

By comparing peak retention times (e.g., relative to 5 alpha-cholestane) in gas chromatograms with those of pure commercial standards, four free fecal neutral sterols and four free fecal acidic sterols were identified. No attempt was made to identify the other peaks in the chromatogram representing keto- and other bile acids.

GLC analysis of neutral and acidic sterols

Gas chromatograph analysis of fecal neutral and acidic sterols was carried out on a Hewlett Packard Gas Chromatograph model 5809. The chromatograph was equipped with a hydrogen flame ionization detector. Hydrogen was used as carrier gas at a flow rate of 2 ml/ min. Neutral and acidic sterols were separated on a 30-m fused silica capillary column (BD-1, inner diameter of 0.32 mm, Chrompack, U.S.A). For optimal separation of the relevant compounds, different temperature programs were selected for neutral and acidic sterols (Czubayko et al., 1991).

The following parameters were used for analysis of neutral steroids:

Temp. Program: 100 °C for 3 min, 150 °C @ 10 °C /min, stay at 270°C for 40 minutes
Inj. Temp: 265°C
Inlet pressure: 11 psi.
FID temp: 325 °C
Split Ratio: 1:1

The following parameters were used for analysis of acidic steroids:

Temp. Program: 3 min at 150°C, 240°C @ 30°C /min (15 min), then 3°C/min to a final temperature of 270°C;
Inj. Temp: 265°C
Inlet pressure: 15 psi
FID temp: 325 °C
Split Ratio: 1:1

Concentrations of different free fecal steroids were calculated from the appropriate neutral steroids (coprostanol, coprostanone, cholesterol, cholestanol, sitosterol, and campesterol, stigmasterol) and acidic steroids (deoxycholic acid, lithocholic acid, chenodeoxycholic acid, cholic acid).

Statistical Analyses

All data were expressed as mean ± SEM. Total fecal steroids, fecal bile acids and fecal neutral sterols were calculated both as 7 day average output (mg/d) (FIG. 8) and as average concentration (mg/g dry fecal weight) (data not shown). Statistical differences were analyzed by analyses of variance and by Scheffe's test utilizing a SAS program.

Fecal Weight

The average daily output of feces (dry weight) at the fourth week was 1.42 ±0.21 g/day on the wheat bran diet; 1.01 ± 0.14 g/day on the oat bran diet; 1.15 ± 0.17g/day on the bamboo shoot diet I and 1.23 ± 0.19 g/day on the bamboo shoot diet II. Wheat bran resulted in a significantly higher daily output of feces. The bamboo shoot diet II had a concentration of insoluble fiber similar to that of the wheat bran fiber, however, the fecal output was relatively lower. This may indicate that bamboo shoot fiber has a different (lower) physiological impact compared to wheat bran fiber. Some studies report that the wheat bran matrix structure rather than the quantity of insoluble hemicellulose is

responsible for the increased fecal bulk (Kritchevsky and Story, 1993). Thus, the data in FIGS. 8-12 indicate that phytosterols rather than insoluble dietary fiber in bamboo shoot have a key impact on fecal weight output.

Fecal neutral steroids

The effects of the 4 diets set forth in FIG. 3 on fecal steroid excretion (average mg/day) and steroid concentration (average mg/g dry weight) in feces are summarized in FIG. 8. Total fecal steroid output (g dry wt/d) was significantly higher in the rats fed the diet supplemented with both dosages of bamboo shoot. With respect to fecal steroid concentration, both bamboo shoot diets led to significantly higher fecal steroid concentration compared with the control diets. Overall, the excretion of feces with lower steroid concentrations occurred in rats supplemented with wheat bran.

As set forth above, data on the total output (mg/d) are shown in FIG. 8. Dietary treatments with bamboo shoots and oat bran all resulted in higher fecal outputs of total neutral steroids compared to rats fed the 15% wheat bran diet. Bamboo shoot diet II (30%) resulted in significantly higher output of neutral steroids. Thus, in the rats fed a bamboo shoot diet, the higher outputs of cholesterol and cholestanol can be attributed to the high fecal neutral steroid output compared to rats fed control diets.

Moreover, it was found that fecal cholesterol increased from 26.0 mg/day to 30.0 mg/day in response to the low (15%) and high (30%) doses of bamboo shoots (data not shown), suggesting that cholesterol is the major neutral steroid excreted. Daily outputs of

both the bacterial metabolites coprostanol and coprostanone on both bamboo shoot diets are significantly higher than that of wheat bran diet, suggesting that other compounds, rather than the insoluble dietary fibers components, play an important role. Coprostanone output is significantly ($p< 0.001$) increased in rats fed oat bran compared to the wheat bran diet and the low dosage bamboo shoot diet. Compared to oat bran diet, the high dosage bamboo shoot diet II had a higher daily output of the bacterial metabolite coprostanone. This indicates that coprostanone is an important neutral steroid of oat bran. The difference in the pattern (levels) of the neutral sterol excretion in different treatment groups indicates that bamboo shoot has a different cholesterol absorption pattern compared to wheat bran and oat bran. This result also suggests that bamboo shoot may have a effect on the microbial floras of the gut compared to oat bran (soluble dietary fiber) and wheat bran (insoluble dietary fiber).

FIG. 9 shows the phytosterol profile of three major plant sterols in feces. Rats in both the bamboo shoot diet I and II groups had a significantly higher output of phytosterols than that of the rats fed the control diets. As the data indicate, fecal sitosterol and campesterol were major components in the feces of the four diet groups. The fecal phytosterol level in bamboo shoot diet II is twice that of bamboo shoot diet II. This increase is dose-dependent. The data indicate that phytosterols in bamboo shoot interfere with neutral steroid absorption. Thus, phytosterols in bamboo shoots play a role in the inhibition of cholesterol absorption.

With respect to fecal steroid concentration (FIG. 10), if fecal phytosterols are

included, both bamboo shoot treatments induce a significantly higher total neutral steroid excretion than wheat bran. The high dosage bamboo shoot diet (30%) led to a high concentration (and output) of fecal cholesterol compared with the control diets. This indicates that the increasing dosages of bamboo shoot significantly inhibit neutral steroid absorption. The wheat bran diet had the lowest fecal neutral steroid concentration compared to the other three diets. This result may be due to the increased levels of insoluble dietary fiber which in return, dilutes the intestinal contents.

Bile Acids

The composition of acidic steroids in feces - primarily bile acids and their derivatives is summarized in FIG. 11. Rats fed both bamboo shoot diets I and II had significantly higher outputs of fecal cholic acid (12.1 and 21.1 mg/d, respectively) as compared to rats fed the control diets. This effect was dose dependent. Rats fed the oat bran diet had the lowest cholic acid output (2.67 mg/d), but had the highest chenodeoxycholic acid fecal output. Rats fed bamboo shoot diet I had a similar pattern of chenodeoxycholic acid fecal output compared to the rats fed the oat bran diet. The chenodeoxycholic acid fecal output (5.4 mg/d) in rats fed bamboo shoot diet II, however, was significantly lower than rats fed bamboo shoot diet I, as well as rats fed the control diets. This indicates that both phytosterol and dietary fiber in bamboo shoots affect bile acid metabolism.

The data further indicate that high dosages of bamboo shoot may affect both

endogenous and exogenous cholesterol. Bamboo shoot at a low dose (15%) may be at a level that just affects exogenous cholesterol.

For secondary bile acid, both bamboo shoot diets I and II and the oat bran diet led to significantly lower lithocholic acid outputs compared to that of the wheat bran treatment. For deoxycholic acid, there was no differences observed among the four diets.

With wheat bran (insoluble fiber) and with oat bran (soluble fiber), there were indications of altered bacterial production of secondary bile acids. Rats fed wheat bran had significantly higher total bile acid output. Both bamboo shoot diets had less bile acid output than that of wheat bran due to less output of secondary bile acids (lithocholic acid and deoxycholic acid). Rats fed bamboo shoot diet II (30%) had a high primary bile acid output, especially cholic acid (80% of total primary acid). This indicates that either cholic acid was poorly absorbed and/or it was excreted from the intestine.

FIG. 12 shows the ratio of secondary bile acids (SBA) to total bile acid (TBA); the chenodeoxycholic acid (CDCA)/cholic acid (CA) ratio and LCA (Lithocholic acid)/DCA (Deoxycholic acid) ratio. Compared to that of wheat bran, the ratio of SBA to TBA is significantly smaller in both bamboo shoot diets I and II. Compared to the oat bran diet, the CDCA/CA ratio was significantly lower in both bamboo diets. There was no significant difference observed among the four diets with respect to the LCA/DCA ratio.

In summary, these data indicate that dietary bamboo shoot in a

hypercholesterolemic rat model significantly increased fecal cholesterol, coprostanol, cholic acid and phytosterol outputs compared with that observed with wheat bran or oat bran fiber control diets. Bamboo shoot diet I (15%) in the diet significantly increased fecal chenodeoxycholic acid output, indicating that it inhibits cholesterol absorption. Bamboo shoot diet II (30%), however, significantly increased cholic acid output, indicating that bamboo shoot affects both cholesterol absorption and hepatic cholesterol synthesis.

The data indicate that phytosterols in bamboo shoot play a key role in causing the hypocholesterolemic effects observed for the bamboo shoot I and II diets. Dietary fiber in bamboo shoot appears to play a minor role in lower cholesterol in the present model. Furthermore, the bamboo shoot diets I and II significantly decreased the ratio of secondary bile acids to total bile acid and the ratio of chenodeoxycholic acid to cholic acid, suggesting that dietary bamboo shoot can benefit colon health.

EXAMPLE 3
Identification and *In Vitro* Evaluation of the Hypocholesterolemic Effect of Bamboo Shoot Phytosterols

The hypocholesterolemic effects of phytosterol extracts isolated from bamboo shoots were evaluated *in vitro* on a human hepatoma cell line (Hep G2) that is an accepted model for studying lipid metabolism, especially the expression of HMG-CoA reductase in human hepatocytes. In particular, bamboo shoots were extracted and fractionated. Certain of these fractions where then added to the Hep G2 cell lines and the expression activity of HMG-CoA reductase mRNA evaluated using RT PCR. The extracted bamboo fractions were also analyzed for content using HPLC techniques.

All the tissue culture reagents including, RPMI 1640, fetal calf serum and antibiotics – antimycotic were purchased from Gibco BRL (Gaithersburg, MD). Restriction enzyme, T4 kinase, superscript II RNAse H^- reverse transcriptase and 5*X* first stand buffer were also purchased from Gibco BRL. Hep G2 cells were purchased from American Type Culture Collection (Rockville, MD). Random primer was from Promega (Madison, WI). All of the chemicals used were purchased from Fisher Scientific (Pittsburgh, PA).

Bamboo Shoots

Canned winter bamboo shoot (*Phyllostachys edulis*) was obtained and processed as set forth in Example 1.

Cell Culture

Hep-G2 cells were cultured in RPMI 1640 containing 10% fetal bovine serum, 292 ug of glutamine/ml, 100 units of penicillin/ml and 100 ug of streptomycin/ml supplemented with 10% fetal calf serum. The cells were incubated at 37 °C under a humidified atmosphere of 95% air and 5 % CO_2.

The cells were seeded at a density of 20-30 x 10^3 /cm^2 and were allowed to attach to the plate for 24 hours. Bamboo shoot fractions (described in more detail below) were applied daily to the cells with a fresh change of media. The control group received the equivalent amount of medium in lieu of a fractionated bamboo shoot sample. All

biochemical assays were performed using a standard format with 3 ml medium per 60 mm tissue culture plate.

Preparation and Fractionation of Bamboo Shoot

The scheme for the bamboo shoot fractionation is shown in FIG. 13. Briefly, 10 grams of dry bamboo shoot powder were homogenized in 300-ml of distilled water for 30 minutes using a POLYTRON homogenizer. The solution was then filtered through a vacuum filter with a coarse porosity (particle retention > 10 um) filter paper. The water fraction was condensed in a rotary evaporator below 40°C. The water extraction (WE) filtrates were stored at 4°C until biological screening (cell culture) and chemical (column chromatography) assay could be carried out.

The remaining residues were further extracted with 300 ml of 100% ethanol for 4 hours. The extract was condensed to 100 ml by rotary evaporation and slightly saponified with 5 ml of 50% KOH, refluxed for 30 min with moderate stirring in a water cooled reflux column on a water bath at 75 °C. This solution was extracted six times with 150 ml of petroleum ether. The petroleum ether extract was condensed by evaporation and dried under N_2. The crude saponified products (total crude fraction or TCE) were further extracted with methanol and methylene chloride according to their polarity. After condensation in a rotary evaporator below 40°C and drying under N_2, two semi-fractions were obtained: total methanol soluble fraction (TMS) and total methanol insoluble, i.e., methylene chloride soluble fraction (TMIS). Fractions TMS and TMIS were further

fractionated by reverse phase and normal phase column chromatography technologies, respectively.

TMS was further fractionated into F1, F2, F3, F4 and F5 fractions and dried under N_2. It was not possible to fractionate and isolate TMIS further because the chemical structures of these compounds were too similar. Overall, 9 fractions were obtained for cell culture screening and GC-MS and LC-MS analysis.

Fractionation of Bamboo Shoot Methanol Soluble Fraction by Column Chromatography and Analysis by HPLC and ApcI-LC-MS

The equipment used for fractionating the bamboo shoots is set forth below:

Varian VISTA 5500 HPLC Solvent Delivery System with a Variable Wavelength UV-VIS Detector, Retriever II Sample Collector and Varian 4290 Integrator

Fractionation Parameters:

Column: Microsorb C18 (Rainin Instrument Co., Inc., MA) dp=5 um,
250 mm x 4.6 mm (i.d.)
Mobile phase: 100% methanol
Wavelength: 210 nm
Flow rate (Pressure): 1.0 mL /min.
Injection vol.: 200 *u*L
Collection: Manually collected every 4.2 minutes as peak eluted

Varian Vista 5500 HPLC Parameters:

Column: Microsorb C18 (Rainin Instrument Co. Inc., MA), dp=5 um, 250 mm x 4.6 mm (i.d.)
Mobile phase: 100% methanol
Wavelength: 210 nm
Flow rate (Pressure): 1.0 mL /min.

Injection vol.: 5 *u*L for LC and LC-MS analysis

VG Platform II Quadrupole Mass Spectr meter parameters:

Masses Scanned (time): 150 – 800 amu (3.00 min.)
Cone Voltage: 10 V
Corona discharge: 3.0 KV
Source temperature: 150 °C
Probe temperature: 450 °C
Analyzer Pressure: 4.3×10^{-5} torr
Mode: AP^+

Fractionation of Bamboo Shoot Methanol Insoluble Fraction (Methylene Chloride Soluble Fraction) by Column Chromatography and Analysis by GC and GC-MS

Equipment Used:

Varian VISTA 5500 HPLC Solvent Delivery System with a Variable Wavelength UV-VIS Detector and a Retriever II Sample Collector

Fractionation Parameters:

Column: Zorbax silica column (Dupont Instruments Co. Inc., DW), *dp* = 5 um, 150 mm x 4.6 mm (i.d.)

Mobile phase and Gradient:

A: Hexane

B: Acetone

Isocratic: 8% B
Flow: 1.0mL/min.
Injection Vol.: 250 uL
Wavelength: 210 nm
Attenuation: 64
Fractions: Manually collected as peak eluted

Varian 3400 GC Parameters:

Column: DB-1, 30 m x 320 (i.d.) capillary column
Temp. Program: 100 °C for 3 min. 300°C @ 10°C/min, stay at 300°C for 20 min
Inj. Temp: 265°C
FID Temp: 325°C
Split Ratio: 1:1

Finnigan MAT 8230 Mass Spectrometer Parameters:

Masses Scanned:	35-550 amu
EI-mode:	70 eV@ 1 mA
GC-MS Interface line:	280°C
MS Inlet Temp.:	240°C
Ion Source Temp.:	280°C

Cell Membrane Sterol Analysis by GC

Preparation of Cell Membrane sterols

Hep G2 cell media was removed and the cells were washed with 2 ml of ice-cold PBS (phosphate buffed saline) 3 times. After removal from the dishes, the cells were suspended into 1 ml of 20-mM mannitol and 2 mM HEPES. The cell suspension was sonicated on ice for 45 seconds at 50% duty cycle. The cell homogenate was stored in small aliquots at –70°C for further analysis.

Measurement of Cell Cholesterol

Sterols were extracted from the membrane homogenates by the method of Bligh and Dyer (1979) and quantified by gas liquid chromatography. An internal standard (5 alpha- cholestane) was added to the cell suspension before extraction. The gas chromatograph (H&P GC 5809) was equipped with a flame ionization detector. The injection port, detector and oven temperatures were maintained at 265 °C, 325 °C and

300 °C, respectively. Data were expressed per mg of membrane protein, which was assayed by the method of Bradford (1976) using Bio RAD Kit (Richmond, CA).

To determine cellular sterol content and composition, aliquots of cell homogenate were saponified for 1 h with 10 ml of 1 N ethanolic NaOH using 5 alpha-cholestane as an internal standard and sterol was extracted by using petroleum ether. The GC parameters were as follows:

Column:	DB-1, 30m x 320 um (i.d.) capillary column
Carrier Gas:	Helium (20 ml/min)
Temp. Program:	100°C for 3 min, 300°C @ 10°C/min, holding at 300 °C for 20 minutes
Injection Temperature:	265 °C
Flame Ion Detection Temperature:	325°C
Split Ratio:	1:20

RT-PCR Determination of the Effect of Bamboo Shoot Crude Extraction on HMG-CoA Reductase mRNA

RT-PCR

The PT-PCR method used in the present invention is based on the method of Tian et al. (1998) with a slight modification. Cells were incubated for three days in RPMI 1640 medium containing bamboo shoot extract. The total RNA was isolated from sample cells, followed by washing and incubation with fresh RPMI 1640 for 15-min (Chomczynski and Sacchi, 1987).

The PCR oligonucleotide primers used are set forth below:

For HMG CoA reductase-1: (GACAATCCTGGAGAAAACGCAC);

For HMG CoA reductase-2: (AGAACACAGCACGGAAAGAAC)

These sequences correspond to the gene-specific primer pairs for human HMG-CoA reductase. Both primer pairs cross introns. Amplification with human genomic DNA as the template yielded no PCR products. The isolated RNA in each sample was transcribed into first strand cDNA under the following conditions: 500 ng random primer, 1*u*g total RNA, 4 *u*L (5*X*) first strand buffer, 3 *u*L bamboo shoot extract, 2 *u*L 10 mM dNTP mixture, 200 Unit SuperScript II RNAse H$^-$ reverse transcriptase. The reaction was incubated at 42 °C for 60 minutes. Equal aliquots of the reverse-transcribed product were amplified in the following PCR reaction containing: 1x Taq buffer, 200 *u*M dNTP, 2*u*L of reverse-transcribed product, 0.2 *u*M each of the primers. The thermal profile consisted of 92°C (45 s), 56°C (60 s), and 72°C (45 s) for 30 cycles. As precautions against contamination, reverse-transcriptase minus and template minus controls were routinely included in the PCR runs.

The PCR products were separated on 1.5% agarose gels and visualized by ethidium bromide staining. The results were recorded on Polaroid Positive/Negative film, and the intensity values were obtained by scanning with a densitometer (BioImage, Ann Arbor, MI).

Statistical Methods

Data were analyzed by a one-way ANOVA. In the case of comparisons, SAS provided Scheffe's multiple comparison test.

Fraction of Crude Bamboo Shoot Extract by LC Normal Phase and LC Reverse Phase

Utilizing HPLC reverse phase chromatography (FIG. 13), the methanol soluble fraction was fractionated into 5 semi crude fractions. The location of these cuts is shown FIG. 13. Fractions within a cut were combined to yield semi-crude fractions and were labeled as fraction 1, 2, 3, 4 and 5, respectively. Each semi-crude fraction was concentrated by rotary evaporation and blown to dryness with nitrogen at room temperature. Fractions 1 and 2 were semisolid yellow oily residues. Fractions 3, 4 and 5 were solid and had a much lighter yellow color.

Further fractionation of the methanol soluble fraction (FIG. 15) (total methylene chloride fraction) was not possible because the fractions were too close to each other to permit collecting cuts.

Determination of the Optimal Dosage and Time Course of Crude Bamboo Shoot Extract on Cell Cholesterol Content

To evaluate the effect of bamboo shoot on Hep G2 cell cholesterol levels and identify active phytochemicals, optimal dosages and time courses were determined using the crude bamboo shoot extract.

To mirror the animal study conditions as closely as possible, the Hep G2 cells were maintained in a serum cholesterol containing media. When crude bamboo shoot extract was applied to Hep G2 cells, a significant reduction of cholesterol content occurred in a

dose dependent manner.

Crude bamboo shoot extract significantly decreased cell cholesterol level at a concentration of 77.5 mg/ml in 3 mL medium (FIG. 16).

As FIG. 17 demonstrates, cell cholesterol content was significantly decreased at day 3 when 3 *u*L bamboo shoot crude extract was applied and at day 2 when 9 *u*L crude bamboo shoot applied. Based on the data set forth in FIGS. 16 and 17, 3 *u*L and 3 days were the optimal conditions chosen for subsequent cell culture evaluations.

The effects of the bamboo shoot fractions on Hep G2 cholesterol concentration (*u*g /mg cell protein) is shown in FIG. 18. The amounts of the different fractions and the physical properties of these fractions were quite different. Each sub-fraction contained substantially equivalent concentrations of bamboo shoot crude extract.

Statistically, five out of nine bamboo shoot (BS) fractions significantly decreased Hep G2 cell cholesterol content. These fractions corresponded to the methanol soluble fraction, the methylene soluble fraction, the total crude bamboo shoot fraction and the methanol soluble sub fractions 1 and 5 at a dosage of 3 *u*L per day. Fraction 2 and Fraction 3 appeared to elevate cell cholesterol levels.

Using GC and LC-MS, the chemical composition of the active components of each of the 5 fractions was partially identified as set forth below.

Primary Determination of Chemical Structure of Five Active Fractions by GC, GC-MS and LC, LC-MS

FIG. 19 shows the HPLC chromatogram of the methanol soluble fraction of bamboo shoot. Many compounds of intermediate polarity are evident.

FIGS. 20 and 21 are the GC chromatograms of total crude bamboo shoot extract and methanol insoluble bamboo shoot extract, respectively. GC and GC-MS analysis results are found in FIGS. 14 and 15, respectively.

These results reveal that bamboo shoot contains a significant amount (2% of dried body weight) of phytosterols. Among these phytosterols, sitosterol and stigmasterol, their derivatives and isomers are major components. As set forth previously, these data represent the first demonstration of the phytosterol profiles in bamboo shoot.

Two GC-MS profiles of total crude bamboo shoot (FIGS. 23 and 21, respectively) and the methylene insoluble bamboo shoot fraction (FIG. 20) indicate that there are significant differences in the lipid profile between the two fractions. In addition to phytosterols, major fatty acids, such as for example, linoleic and palmitic acid are present in bamboo shoot (FIG. 20). It is believed that these phytosterols exist in the ester form.

Two GC-MS profiles of total crude bamboo shoot (FIG. 21) and the methylene insoluble bamboo shoot fraction (FIG. 20) indicate that there are significant differences in the lipid profile between the two fractions. In addition to phytosterols, major fatty acids, such as linoleic and palmitic acid, are present in bamboo shoot. Again, it is believed that

these phytosterols exist in the ester form.

The mass spectra of F1 is shown in FIG. 24. Even though it was not possible to fully characterize these compositions, it was evident that they were phytosterols. For example, F1 is a phytosterol with M^+ at 414(100%), the major peaks are at 55 (56%), 81 (35%), 95 (30%), 218 (10%), 234 (2.9%), 313 (3.5%), 396 (1.9%) and 414 (2.5%). F5 (FIG. 25) is a phytosterol with M^+ is at 414 (30%), the major peaks are at 396 (20%), 385 (18%), 313 (45%), 133 (64%), 95 (50%), 75 (68%), 43 (100%).

Surprisingly, only the total crude bamboo shoot extract significantly down regulated the HMG-CoA reductase mRNA transcription (FIG. 26). These results indicate that the bamboo shoot mixture, rather than any single fraction, down regulated HMG-CoA reductase. Other fractions, however, such as for example, the methanol soluble fractions or the methylene chloride fraction may be affecting other enzymes in the cholesterol synthesis pathway.

In summary, the mass spectra data indicate that the identified compounds are a mixture of plant sterols, oxygenated sterols and their ketone and aldehyde metabolites. The active compounds are likely a series of ester forms of phytosterols. Only the crude bamboo shoot extract (with both methanol soluble fraction and methylene chloride soluble fraction) significantly decreased the cholesterol content (synthesis) in the Hep G2 cell line while significantly down regulating HMG-CoA reductase mRNA expression.

significantly decreased the cholesterol content (synthesis) in the Hep G2 cell line while significantly down regulating HMG-CoA reductase mRNA expression.

The methanol soluble fraction (less polar) and the methylene chloride soluble fraction (more non-polar fraction) both decreased the cell cholesterol level but had no effect on cell HMG-CoA reductase mRNA expression.

The polar (water fraction) fraction had no effect on Hep G2 cell cholesterol activity. One of the chemical differences between the crude bamboo shoot extract and the methanol soluble fraction /methylene chloride soluble fraction was the presence of ester forms of the sterol in the methanol soluble fraction. In the methylene chloride soluble fraction, phytosterols exist in the hydrophobic form. In the methanol soluble fraction, part of the sterols likely exist as an ester form (MSF1) and part of the sterols (methanol soluble fraction F5) exist as a straight hydrophobic form.

These results show that bamboo shoot is an excellent source of phytosterols. Activity for the present compositions has been demonstrated both *in vivo* (rat studies), as well as *in vitro* (Hep G2 studies). Bamboo shoot non saponifiable sterols in the methanol soluble fraction and the methylene chloride soluble fraction have hypocholesterolemic properties. Through column fractionation and GC and LC-MS techniques, the effective compounds were identified and confirmed to be phytosterols. No effect upon HMG-CoA reductase transcription level, however, was observed for the individual fractions tested. The down regulation of HMG-CoA reductase was surprisingly only observed in the crude

bamboo shoot fraction, indicating that extracts of bamboo shoot may be the preferred hypocholesterolemic agent compared with fractions thereof.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention and all such modifications are intended to be included within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A composition for reducing cholesterol levels in a mammal comprising a phytosterol-containing extract isolated from bamboo shoot.

2. The composition of claim 1, wherein said cholesterol levels include serum total cholesterol, LDL cholesterol and total liver lipids.

3. The composition of claim 2, wherein said total liver lipids include liver cholesterol and liver triglycerides.

4. The composition of claim 1, wherein said extract is a crude extract.

5. The composition of claim 1, wherein said extract comprises one or more fractions containing one or more phytosterols in each of said fractions.

6. The composition of claim 1, wherein said extract further comprises sitosterol, sitostanol, stigmasterol and derivatives and isomers thereof.

7. The composition of claim 1, wherein said extract further comprises a mixture of phytosterols including beta-sitosterol, stigmasta-3,5-dien-7 one, stigmast-4-en-3-one, stigmasta-5,22-dien-3-ol, campesterol, and derivatives and isomers thereof.

8. The composition of claim 1, further comprising chemically modified forms of phytosterols including esterified, glycosidic, saturated or unsaturated and oxysterol

forms thereof.

9. The composition of claim 1, wherein said bamboo shoot belongs to the family *Gramineae*.

10. The composition of claim 1, wherein said bamboo shoot is selected from the group consisting of *Bambusa oldhami Nakai, Bambusa edulis, Pseudosas usawai, Zizania latiflia, Saccharum officinarum, Dendrocalamus latiflorus* Munro, *Phyllostachys edulis, Phyllostachys pubescens*, and *Phyllostachys makinoi*.

11. A pharmaceutical composition comprising an effective cholesterol-lowering amount of the extract of claim 1 together with a pharmaceutically acceptable carrier.

12. A dietary supplement comprising as an active agent a cholesterol lowering amount of a phytosterol-containing extract isolated from bamboo shoot.

13. The dietary supplement of claim 12, wherein said extract lowers serum total cholesterol, LDL cholesterol and total liver lipids.

14. The dietary supplement of claim 13, wherein said total liver lipids include liver cholesterol and liver triglycerides

15. The dietary supplement of claim 12, wherein said extract is a crude extract.

16. The dietary supplement of claim 12, wherein said extract contains one or more phytosterols.

17. The dietary supplement of claim 12, wherein said extract further comprises sitosterol, sitostanol, stigmasterol and derivatives and isomers thereof.

18. The dietary supplement of claim 12, wherein said extract further comprises a mixture of phytosterols including beta-sitosterol, stigmasta-3,5-dien-7 one, stigmast-4-en-3-one, stigmasta-5,22-dien-3-ol, campesterol, and derivatives and isomers thereof.

19. The composition of claim 12, wherein said extract further comprising chemically modified forms of said phytosterols including esterified, glycosidic, saturated or unsaturated and oxysterol forms of said thereof.

20. A pharmaceutically useful composition comprising an extract containing one or more phytosterols isolated from bamboo shoots.

21. The pharmaceutical composition of claim 20, further including pharmaceutically acceptable derivatives and salts of said extract.

22. The pharmaceutical composition of claim 20, further comprising chemically modified forms of said phytosterols including esterified, glycosidic, saturated or unsaturated and oxysterol forms of said phytosterols.

23. A method for lowering cholesterol levels in a mammal comprising administering to said mammal a composition comprising an effective amount of a phytosterol-containing extract isolated from bamboo shoots sufficient to lower said cholesterol levels.

24. A method of making a composition for lowering cholesterol levels in a mammal comprising:

1) obtaining an extract of phytosterols from a source of bamboo shoots; and

2) combining said extract with a suitable delivery vehicle for administering cholesterol-lowering amounts of said extract to said mammal.

25. A method of inhibiting cholesterol absorption and/or increasing fecal excretion of neutral and acid steroids in a mammal comprising administering to said mammal an effective amount of a composition having as its primary active agent one or more phytosterols isolated as an extract from bamboo shoots.

26. The method of claim 25, wherein said composition is a pharmaceutical or a dietary supplement.

27. The method of claim 25, wherein composition decreases serum total and LDL cholesterol and total liver lipids in said mammal.

28. The method of claim 27, wherein said total liver lipids further includes liver cholesterol and liver triglycerides.

29. A method of inhibiting cholesterol synthesis by administering to a mammal a cholesterol inhibiting amount of a composition comprising an extract containing one or more phytosterols isolated from bamboo shoot.

30. A method for reducing cholesterol levels in a mammal comprising in combination inhibiting cholesterol absorption and inhibiting cholesterol synthesis by administering to said mammal an effective amount of a composition comprising an extract of one or more phytosterols isolated from bamboo shoot.

31. The method of claim 30, wherein said composition inhibits said cholesterol synthesis by suppressing or decreasing expression of one or more enzymes in the cholesterol synthesis pathway.

32. The method of claim 31, wherein said composition inhibits 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase mRNA activity.

33. The composition of claim 30, wherein said composition is a pharmaceutical or dietary supplement in combination with a suitable carrier.

34. A method for lowering cholesterol levels in a mammal by reducing or

inhibiting cholesterol synthesis and cholesterol absorption comprising administering to said mammal cholesterol lowering amounts of bamboo shoots.

35. The method of claim 34, wherein said bamboo shoots are formed into a powder and combined with a food source, a dietary supplement or a pharmaceutical composition.

36. The method of claim 34, wherein bamboo shoots are fresh.

37. The method of claim 34, wherein said bamboo shoots are dried.

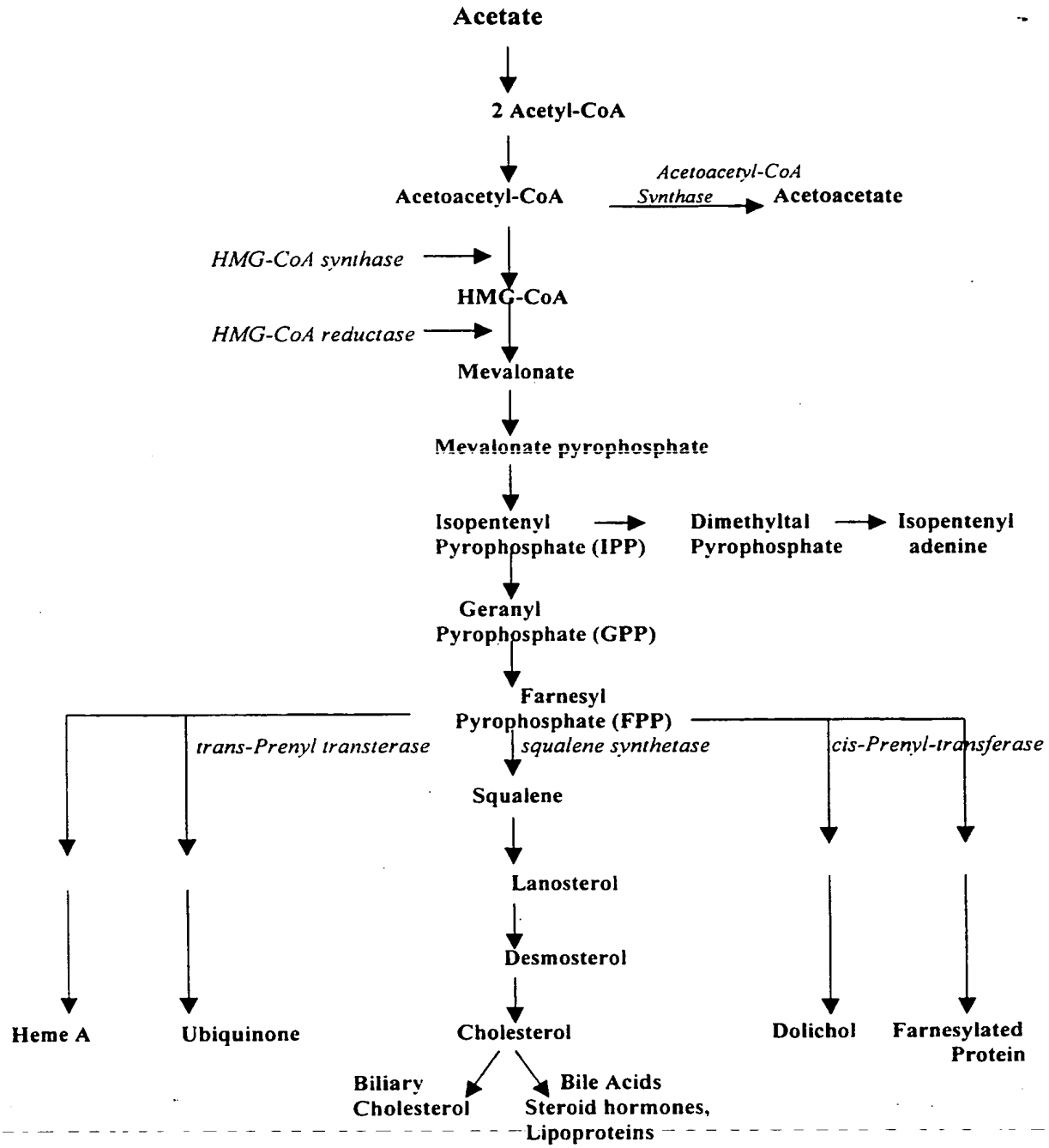

Acetate
2 Acetyl-CoA
Acetoacetyl-CoA
Acetoacetyl-CoA Synthase
Acetoacetate
HMG-CoA synthase
HMG-CoA
HMG-CoA reductase
Mevalonate
Mevalonate pyrophosphate
Isopentenyl Pyrophosphate (IPP)
Dimethyltal Pyrophosphate
Isopentenyl adenine
Geranyl Pyrophosphate (GPP)
Farnesyl Pyrophosphate (FPP)
trans-Prenyl transferase
squalene synthetase
cis-Prenyl-transferase
Squalene
Lanosterol
Desmosterol
Heme A
Ubiquinone
Cholesterol
Dolichol
Farnesylated Protein
Biliary Cholesterol
Bile Acids
Steroid hormones,
Lipoproteins

Figure 1

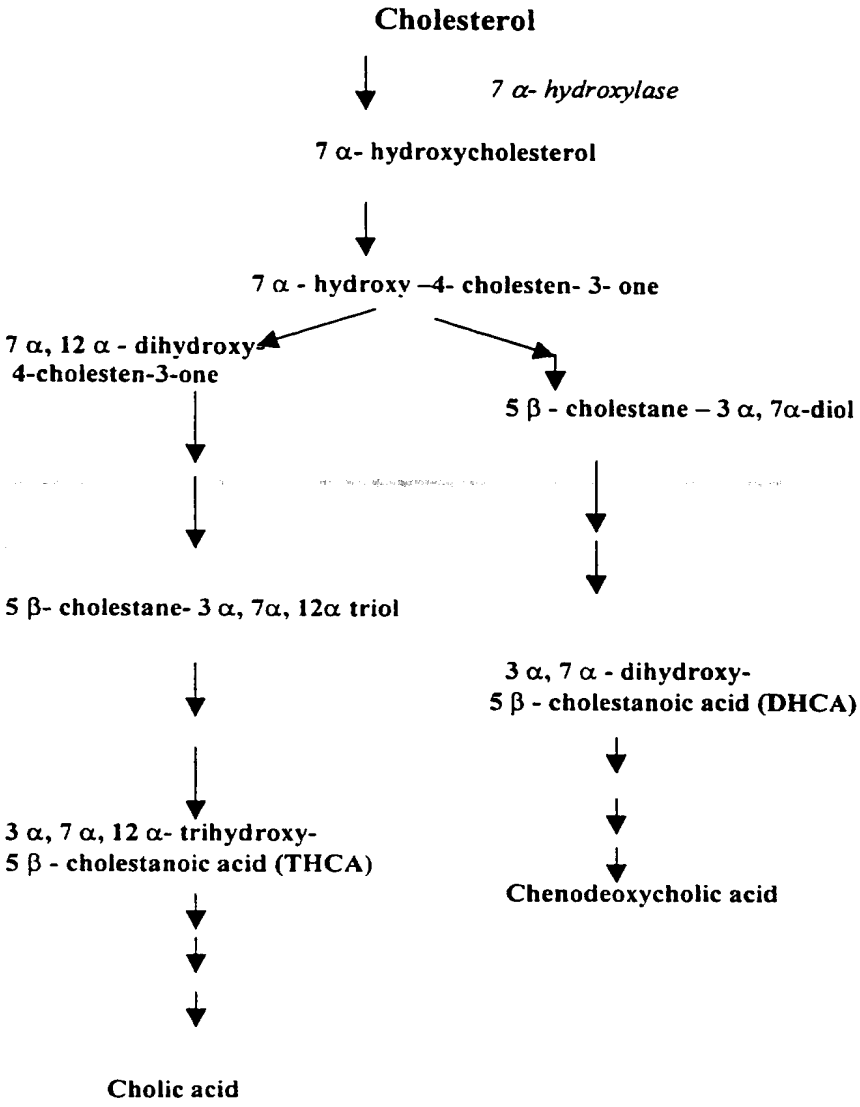

Cholesterol
7 α- hydroxylase
7 α- hydroxycholesterol
7 α - hydroxy –4- cholesten- 3- one
7 α, 12 α - dihydroxy-
4-cholesten-3-one
5 β - cholestane – 3 α, 7α-diol
5 β- cholestane- 3 α, 7α, 12α triol
3 α, 7 α - dihydroxy-
5 β - cholestanoic acid (DHCA)
3 α, 7 α, 12 α- trihydroxy-
5 β - cholestanoic acid (THCA)
Chenodeoxycholic acid
Cholic acid

Figure 2

Diet Ingredient	Wheat Bran Diet	Oat Bran Diet	Bamboo Shoot Diet	
			I	II
----------g./Kg. ----------				
Casein	200	200	200	155
DL-methioine	3	3	3	3
Maltodextrin 10	75	75	75	75
Corn Starch	225	225	225	152
Sucrose	200	200	200	200
Corn Oil	100	100	100	91
Salts S10001	40	40	40	40
Vitamin V1000	10	10	10	10
Choline Bitartrate	2	2	2	2
Cholesterol	10	10	10	10
Sodium Cholate	1	1	1	1
BS. Test material	0	0	150	300
Wheat Bran	150	0	0	0
Oat Bran	0	150	0	0

Figure 3

	Dietary Treatments			
	Wheat Bran	Oat Bran	Bamboo Shoots I	Bamboo Shoots II
TC (mg/dl)	128.7±4.7[a]	117.9±4.5[a]	82.0±3.9[b]	64.5±3.6[c]
LDL-C (mg/dl)	89.2± 5.5[a]	82.4±5.7[a]	42.5±3.0[b]	25.5± 2.6[c]
Triglycerides (mg/dl)	26.4± 1.0[ab]	25.4±1.1[b]	28.1±1.0[a]	28.7±1.1[a]
HDL-C (mg/dl)	52.7±5.9[a]	50.8±6.2[a]	55.9±5.4[a]	54.7±5.7[a]
HDLC/LDLC	0.29 ±0.15[c]	0.30± 0.16[c]	0.66 ± 0.33[b]	1.21±0.41[a]

Figure 4

	Dietary Treatments			
	Wheat Bran	Oat Bran	Bamboo Shoots I	Bamboo Shoots II
Liver wt(g)/100g body wt	6.37±0.32 [a]	6.49±0.40 [a]	6.18±0.30 [a]	5.38±0.31 [b]
Liver lipids(g)/100g of liver	26.6±4.0 [a]	24.0±5.0 [ab]	19.0±4.2 [bc]	17.8±2.0 [c]
Liver cholesterol (mg/g)	76.3± 10.1[a]	70.2± 9.9[a]	46.0± 9.5[b]	24.0± 7.4[c]
Liver Triglycerides (mg/g)	63.9±7.8 [a]	64.5±6.5 [a]	63.4±5.2 [a]	43.6±4.7 [b]

Figure 5

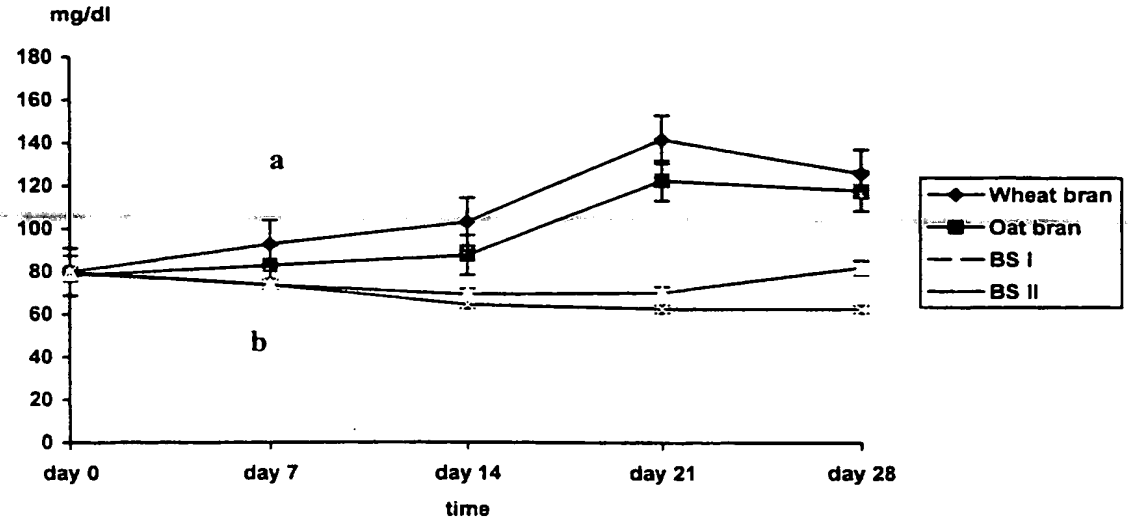


Figure 6

	Dietary Treatments			
Composition	**Wheat Bran**	**Oat Bran**	**Bamboo Shoots**	
			I	**II**
TDF (g/100g)	6.6	1.8	3.7	7.4
IDF (g/100g)	6.6	0.9	3.5	7.0
SDF (g/100g)	0	0.9	0.2	0.4
Phytosterols (mg/day)*				
β-sitosterol	trace	trace	6.1	12.2
Campesterol	trace	trace	3.4	6.8
Stigmsta-5,22-Dien-3-ol	trace	trace	1.0	2.0

Figure 7

	Dietary Treatments			
Steroids	**Wheat Bran**	**Oat Bran**	**Bamboo Shoots** I	II
Neutral Steroids				
mg/day	28.85±2.61 [d]	50.71±5.07[c]	56.79± 5.46[b]	71.42±6.69 [a]
Acid Steroids				
mg/day	46.79± 3.95 [a]	36.51±2.83 [c]	43.61± 3.76[b]	44.54± 4.41[b]
Total steroids				
mg/day	75.64±6.56 [d]	87.22±7.90 [c]	100.40±9.22[b]	115.96±11.10 [a]

Figure 8

	Dietary Treatments			
Sterols	**Wheat Bran**	**Oat Bran**	**Bamboo Shoots**	
			I	**II**
Phytosterols		*mg/day*		
Campesterol	0.84±0.11 [d]	1.32±0.11 [c]	2.6±0.29 [b]	5.50±0.49 [a]
Stigmasterol	0.18±0.01 [d]	0.23±0.02 [c]	0.44±0.03 [b]	0.64±0.04 [a]
Beta-sitosterol	2.12±0.19 [d]	2.51±0.26 [c]	4.37±0.41 [b]	7.41±0.58 [a]
Total (above) phytosterols *	3.14±0.31 [d]	4.06±0.39 [c]	7.41±0.73 [b]	13.55±1.11 [a]
Total neutral steroids**	28.85±2.61 [d]	50.71± 5.07 [c]	56.79±5.46 [b]	71.42±6.69 [a]

Figure 9

	Dietary Treatments			
Steroids	**Wheat Bran**	**Oat Bran**	**Bamboo Shoots** I	II
Neutral steroids	*mg/day*			
Cholesterol	17.91±1.60 [c]	18.13±2.00 [c]	25.92±2.71 [b]	30.02±3.30 [a]
Cholesteranol	0.57±0.07 [d]	10.69±1.19 [a]	4.82±0.49 [c]	6. 61±0.68 [b]
Coprostanol	6.17±0.52 [c]	14.11±1.21 [b]	16.03±1.22 [ab]	17.9±1.32 [a]
Coprostanone	1.06±0.11 [c]	3.72± 0. 28 [a]	2.61±0.31 [b]	3.34±0.25 [ab]
Total non-phyto neutral steroid*	25.71±2.30 [c]	46.65±4.68 [b]	49.38±4.73 [b]	57.87±5.55 [a]

Figure 10

Acid Steroids	Dietary Treatments			
	Wheat Bran	Oat Bran	Bamboo Shoots I	Bamboo Shoots II
	mg/day			
Primary bile acids				
Cholic acid	6.77±0.53 [c]	2.67±0.20 [d]	12.12±1.06 [b]	21.14±1.92 [a]
Chenodeoxycholic acid	10.63±1.03 [c]	14.92±1.31 [a]	12.53±1.08 [b]	5.42±0.54 [d]
Subtotal	17.40±1.56 [b]	17.59±1.51 [b]	24.65±2.14 [a]	26.56±2.46 [a]
Secondary bile acids				
Lithocholic acid	17.29±1.31 [a]	10.78±0.83 [b]	9.92±0.88 [b]	9.82±1.03 [b]
Deoxycholic acid	12.10±1.08 [a]	8.14±0.49 [b]	9.04±0.74 [b]	8.16±0.82 [b]
Subtotal	29.39±2.39 [a]	18.92±1.32 [b]	18.96±1.62 [b]	17.98±1.85 [b]
Total acid steroids	46.79±3.95 [a]	36.51±2.83 [c]	43.61±3.76 [b]	44.54±4.41 [b]

Figure 11

	Dietary Treatments			
Ster ids	Wheat Bran	Oat Bran	Bamboo Shoots	
			I	II
SBA/TBA*	0.63 [a]	0.48 [b]	0.43 [b]	0.40 [b]
CDCA/CA**	1.57 [b]	5.59 [a]	1.03 [c]	0.26 [d]
LCA/DCA***	1.45 [a]	1.33 [a]	1.10 [a]	1.20 [a]

Figure 12

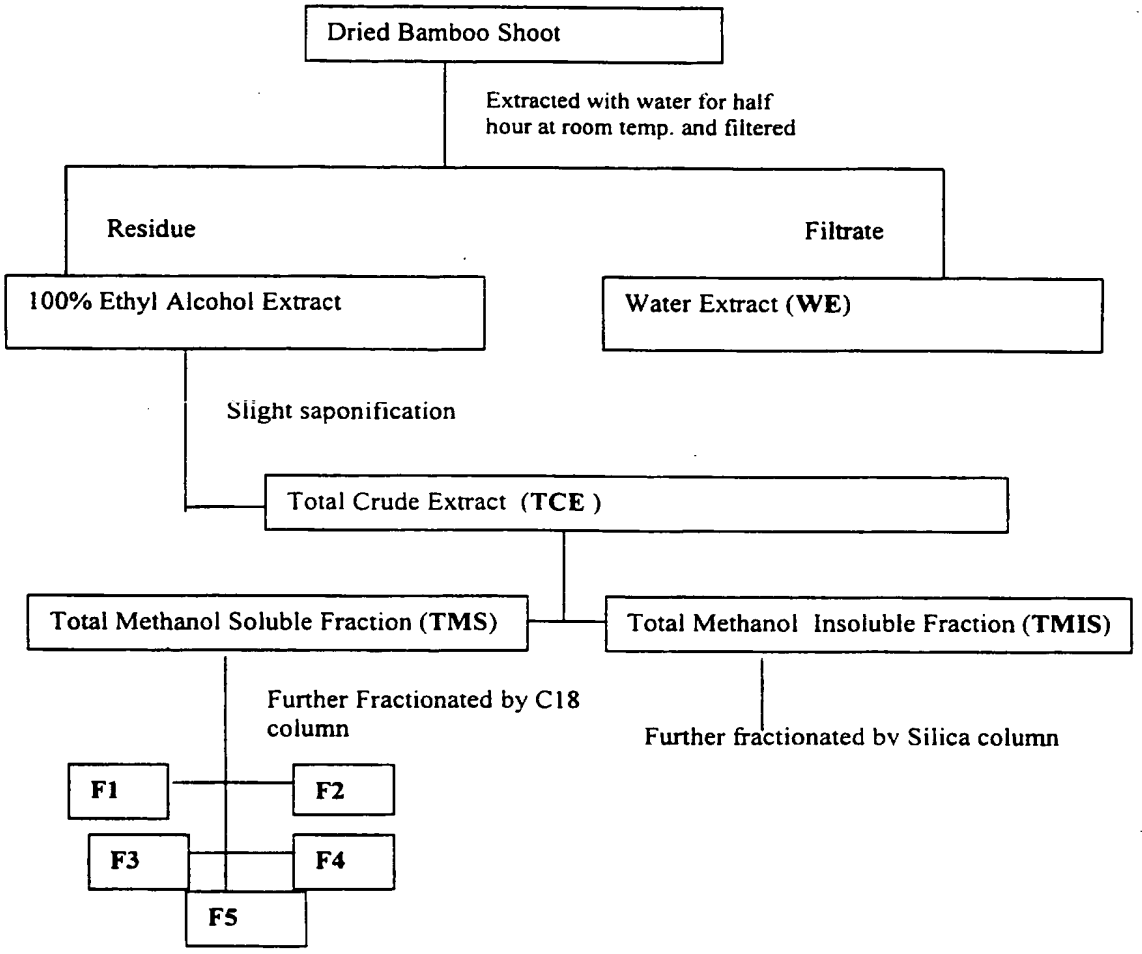


Figure 13

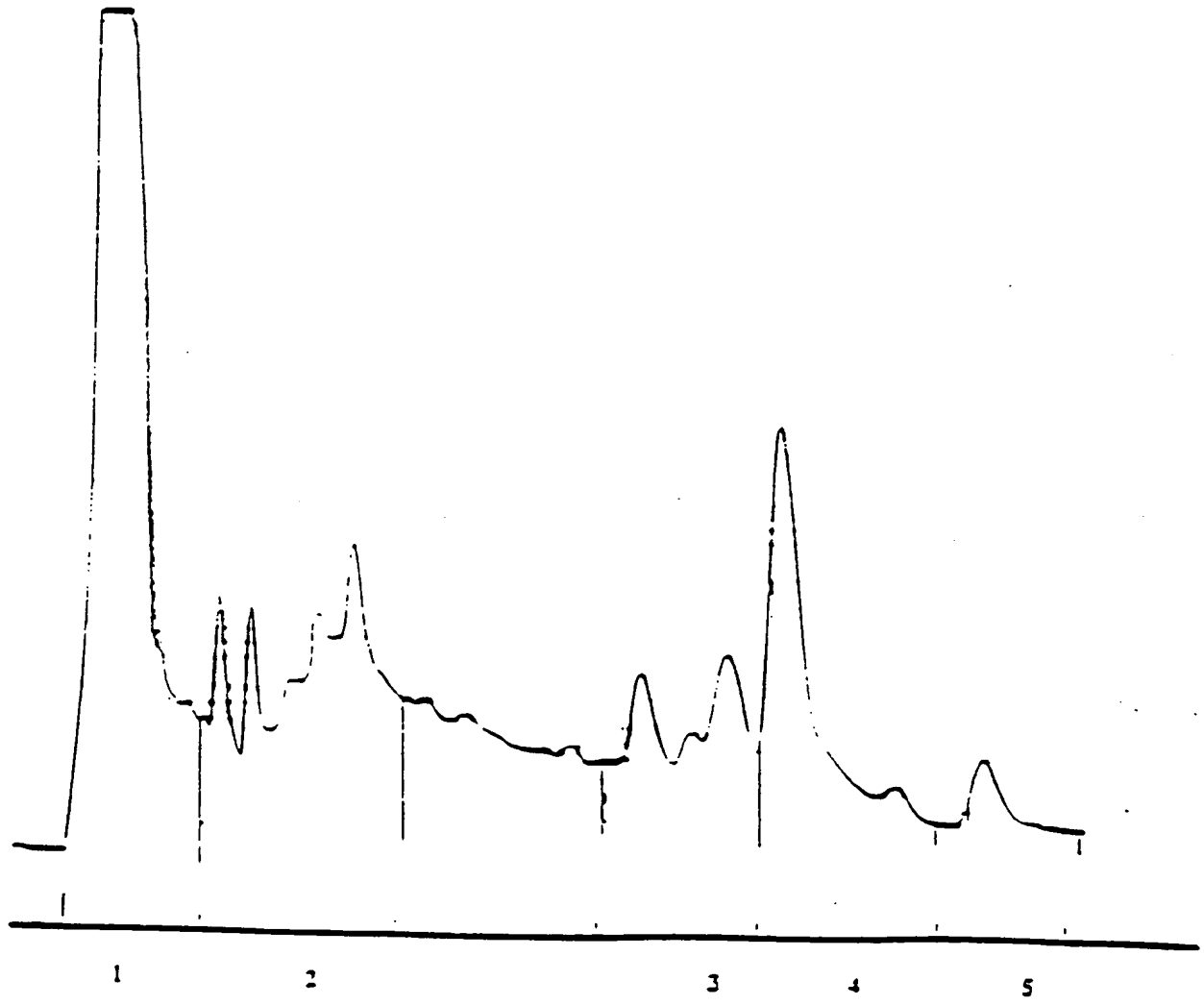


Figure 14

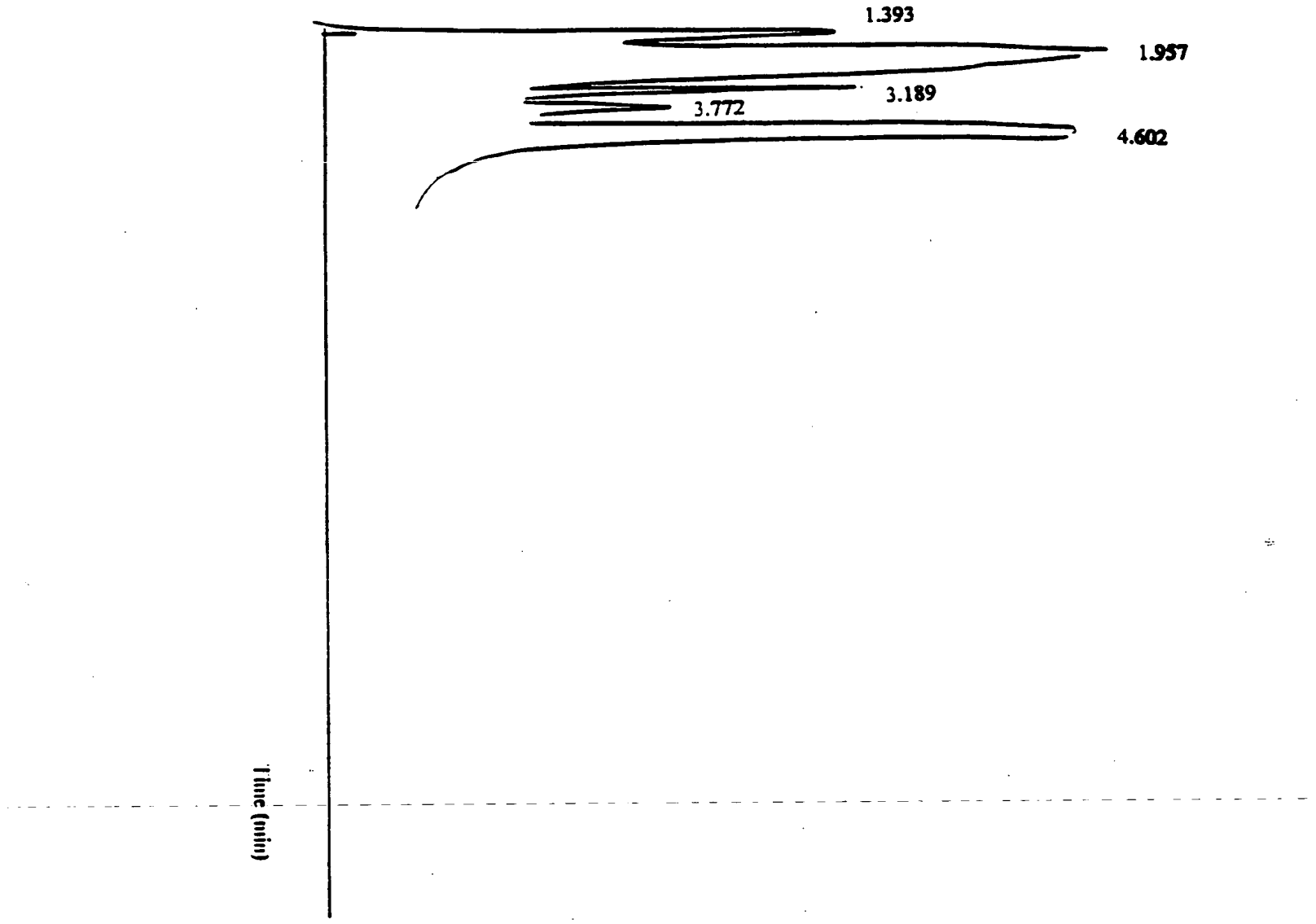


Figure 15

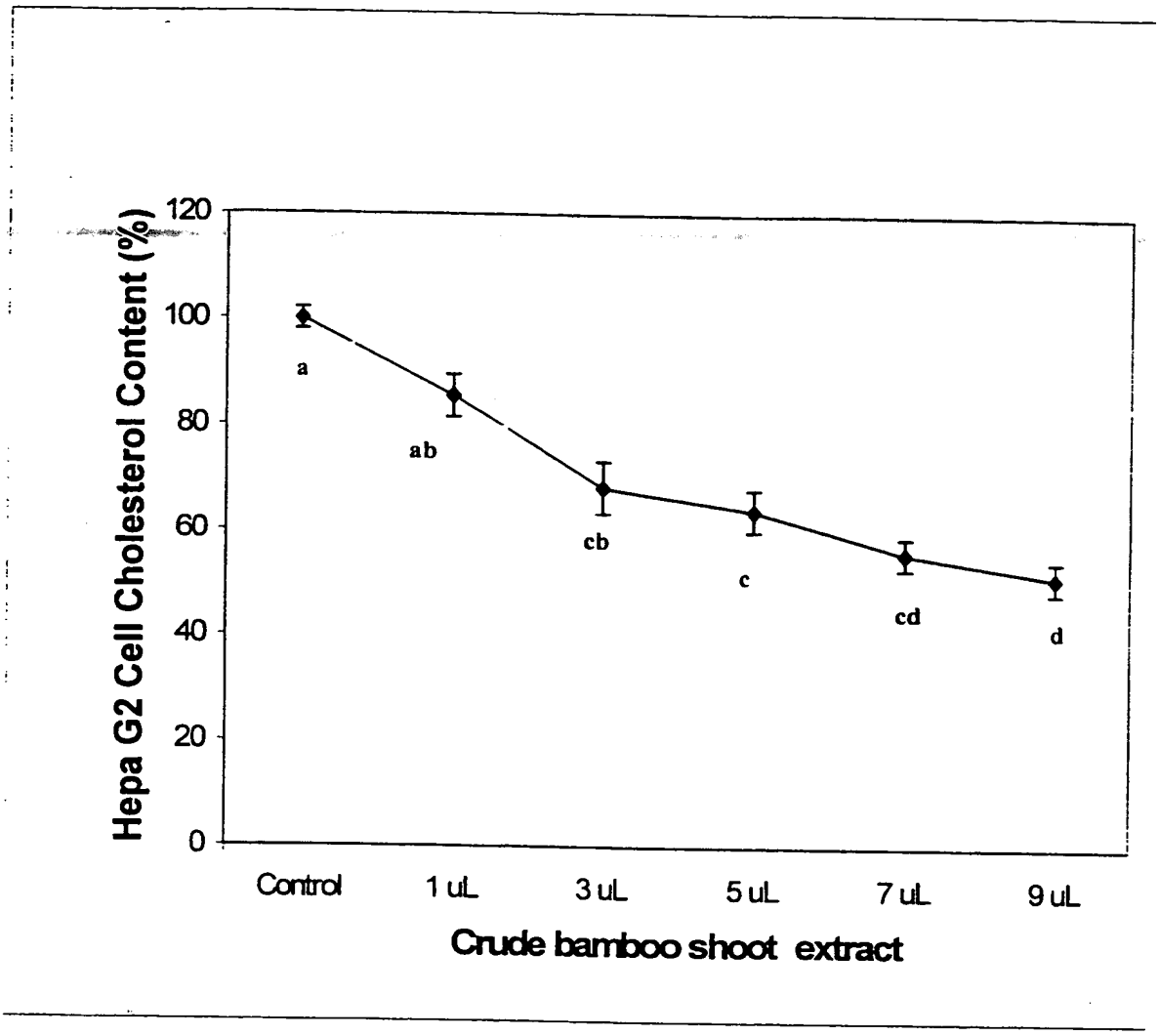


Figure 16

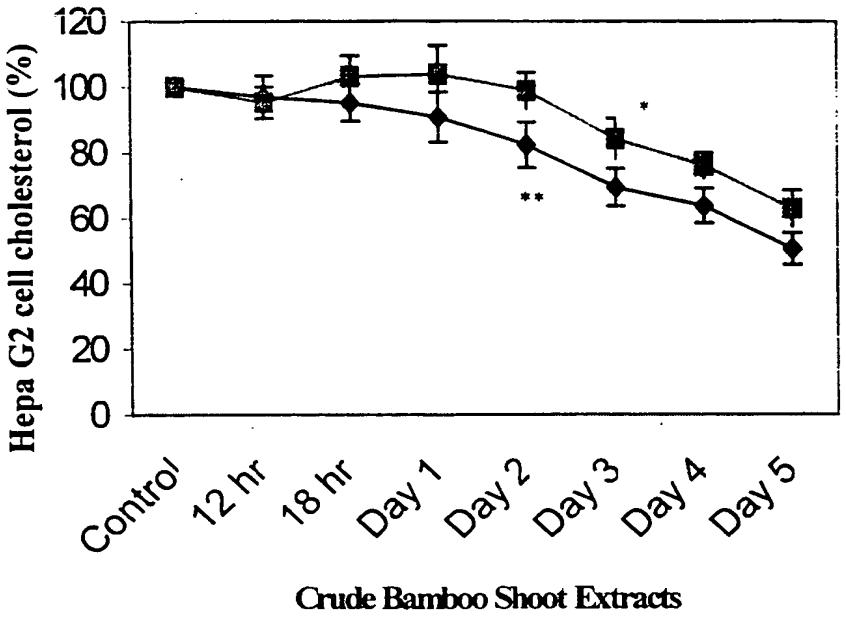


Figure 17

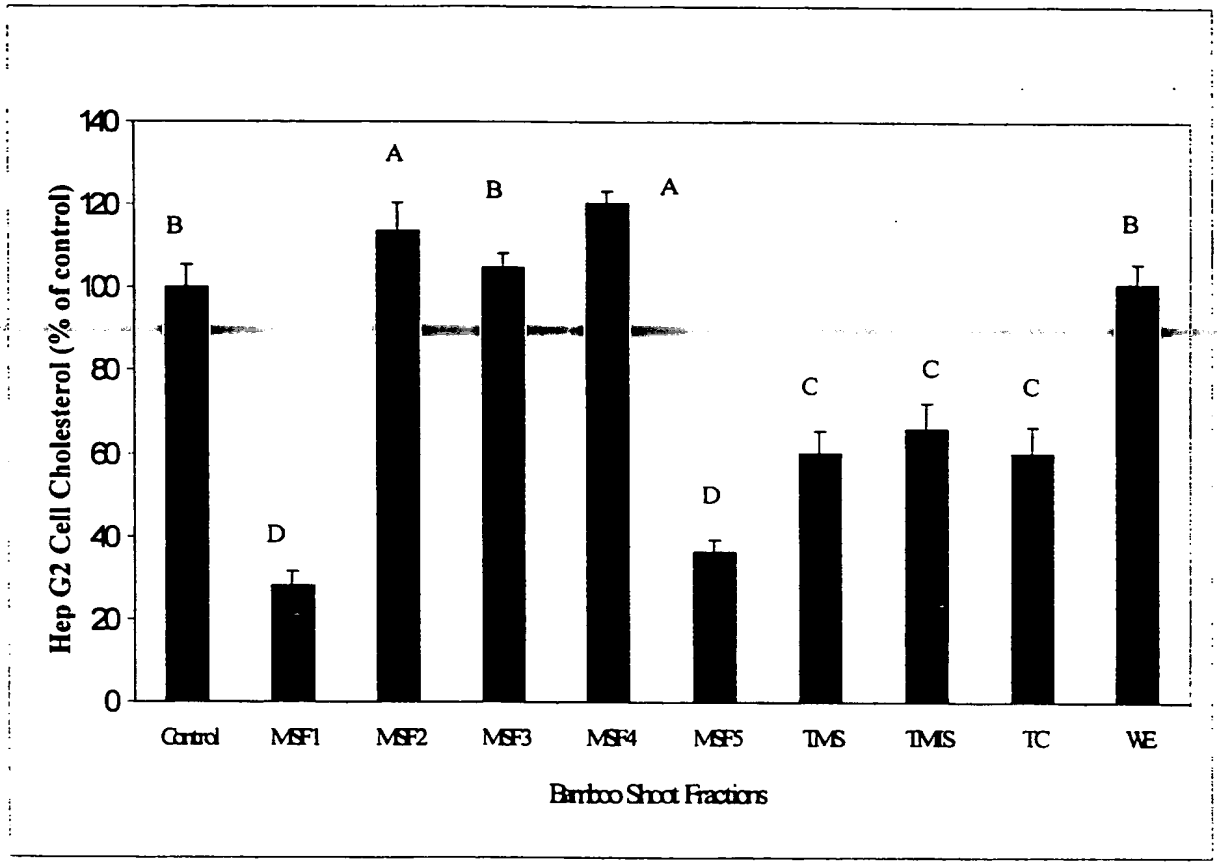
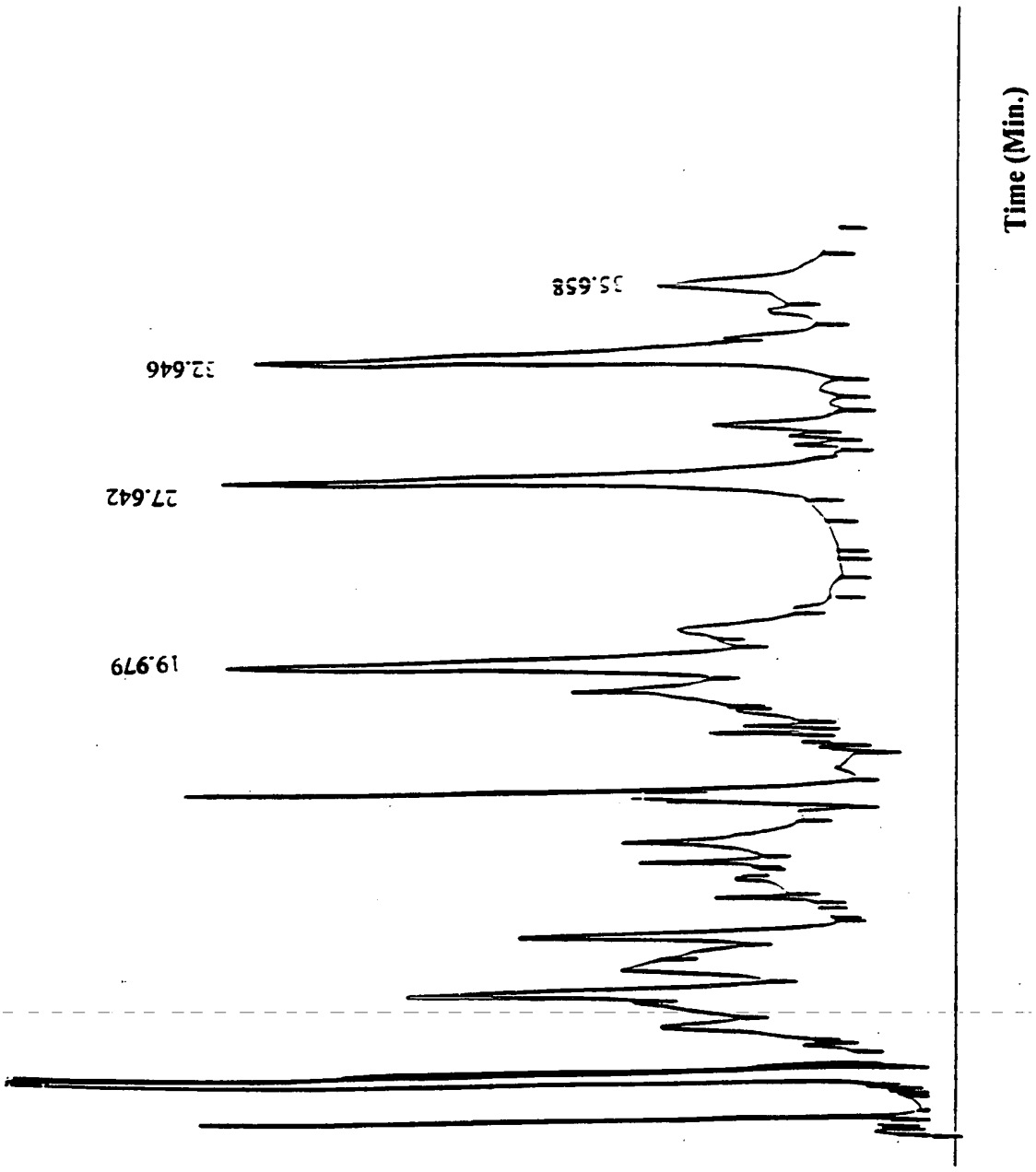


Figure 18

Figure 19



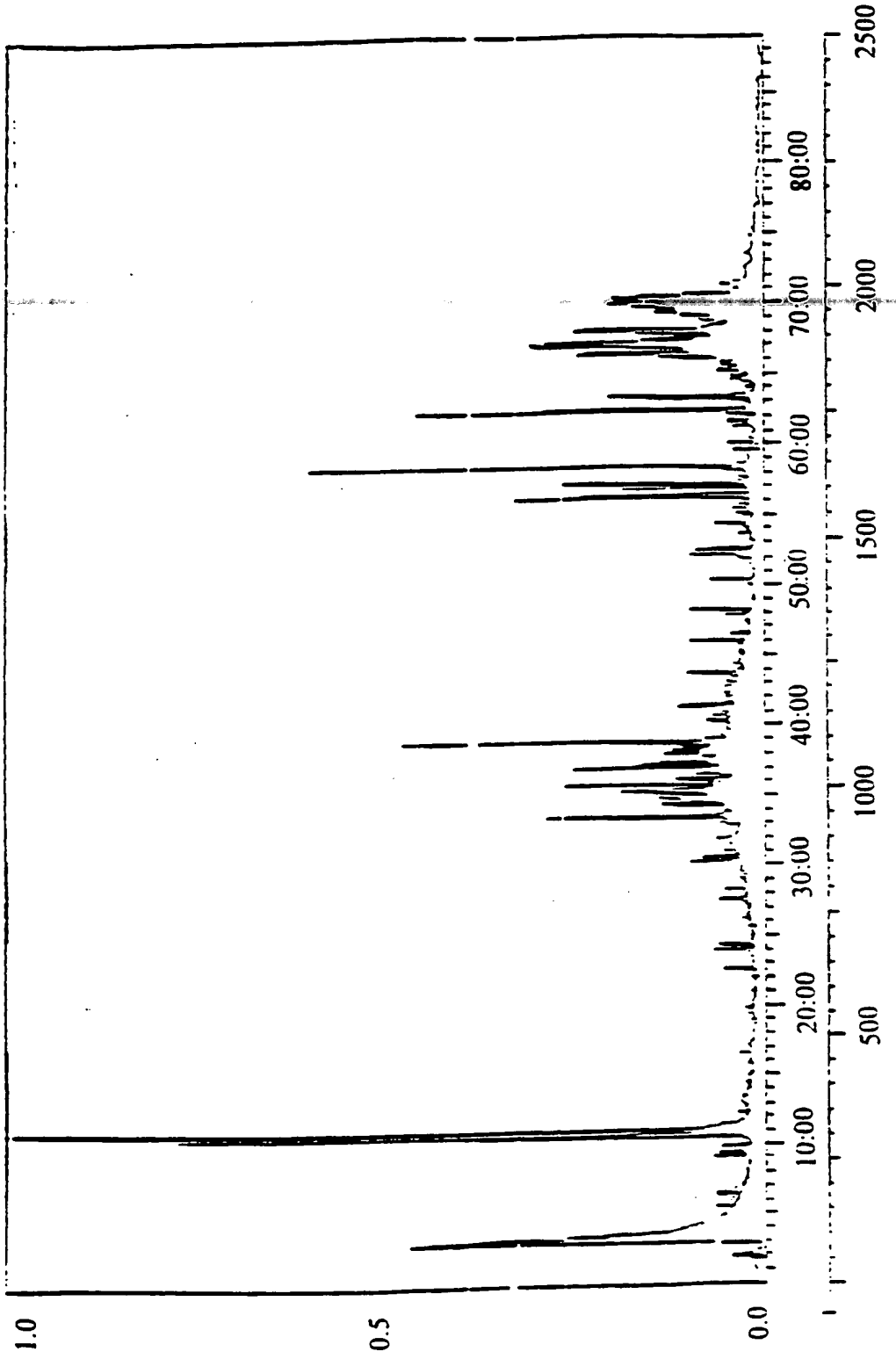
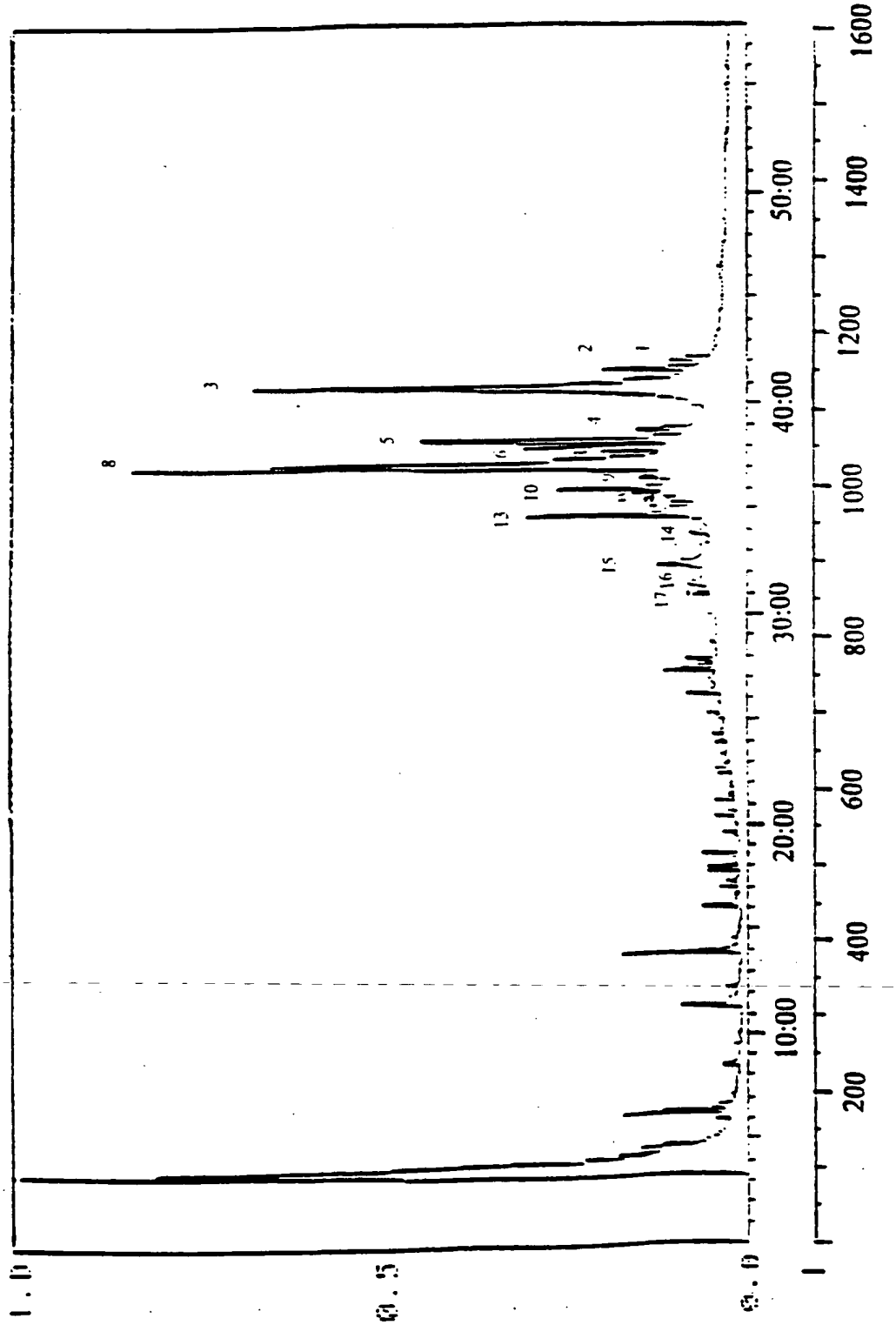


Figure 20


1.0
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200
400
600
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16
17

Figure 21

Name of the Compound	Number of the peak	Spect #	MW	ppm
Unknown sterol	1	1162	426	188.2
Stigmasa-3,5-Dien- 7 one	2	1150	410	295.1
Stigmast-4-en-3-one	3	1129	412	1925.8
Unknown sterol	4	1071	396	220.2
Unknown sterol	5	1059	414	1281.9
Unknown sterol	6	1048	398	668.2
Possible sitostanol	7	1031	416	368.2
Beta-sitosterol	8	1025	414	1658
Isomer of beta-sterol	9	1011	414	121.6
Unknown sterol	10	995	416	349.8
Unknown sterol	11	990	400	165.1
Stigmasta-5,22 –Dien-3-ol	12	981	412	140.4
Campesterol	13	960	400	777.4
Unknown sterol	14	937	402	65.2
Unknown sterol	15	896	396/400	198.4
Unknown sterol	16	892	396/400	108.9
Unknown sterol	17	870	396	71.34

Figure 22

Name of the Compound	Peak No.	MW	Major Peak in Mass Spectrum
Unknown sterol	1	426 (20%)	411 (10%), 313 (8%), 303 (12%), 285 (11%), 221(10%), 175 (17%), 163 (35%), 138 (56%), 123 (75%)
Stigmasa-3,5-Dien- 7 one	2	410 (56%)	395 (8%), 275 (18%), 269 (24%), 227 (12%), 213 (10%), 136 (69%)
Stigmast-4-en-3-one	3	412 (58%)	397 (10%), 370 (18%), 327 (9%), 289 (32%), 271 (14%), 229 (47%), 124 (100%)
Unknown sterol	4	396 (22%)	381 (8%), 367 (11%), 298 (16%), 269 (25%), 231 (18%)
Unknown sterol	5	414 (72%)	399 (15%), 246 (10%), 231(100%), 217 (30%)
Unknown sterol	6	398 (40%)	383 (9%), 356 (12%), 313 (8%), 275 (22%), 229 (38%), 124 (100%)
Possible sitostanol	7	416 (39%)	401 (37%), 381(11%), 329 (16%), 233 (24%), 215 (40%)
Beta-sitosterol	8	414 (100%)	396 (35%), 381 (24%), 329 (46%), 303 (26%), 273 (19%), 255 (20.5), 231 (31%), 213 (31%)
Isomer of beta-sterol	9	414 (24%)	396 (42%), 381 (30%), 354 (12%), 329 (12%), 276 (18%), 255 (25%), 213 (27%)
Unknown sterol	10	416 (55%)	398 (56%), 383 (20%), 344 (19%), 233 (40%), 215 (100%)
Unknown sterol	11	400 (37.5%)	385 (10%), 231 (100%), 217 (21%), 213 (15%), 189 (10%)
Stigmasta-5,22 –Dien-3-ol	12	412 (24%)	394 (6%), 351 (10%), 300 (16%), 271 (24%), 255 (26%), 213 (10%)
Campesterol	13	400 (24%)	382 (18%), 367 (13%), 315 (16%), 273 (12%), 255 (14%), 231(22%), 213 (21%)
Unknown sterol	14	402 (22%)	384 (19%), 369 (15%), 276 (9%), 233 (20.5), 215 (56%)
Unknown sterol	15	396/400 (22%)	381 (5%), 354 (4%), 281 (10%), 217 (45 %), 147 (24%), 97 (64%)
Unknown sterol	16	396/400 (37%)	381 (9%), 288 (13%), 283 (11%), 255 (18%), 213 (14%), 207 (20%)
Unknown sterol	17	396 (26%)	383 (12%), 341 (15%), 281 (12%), 255 (15%), 215 (20%)

Figure 23

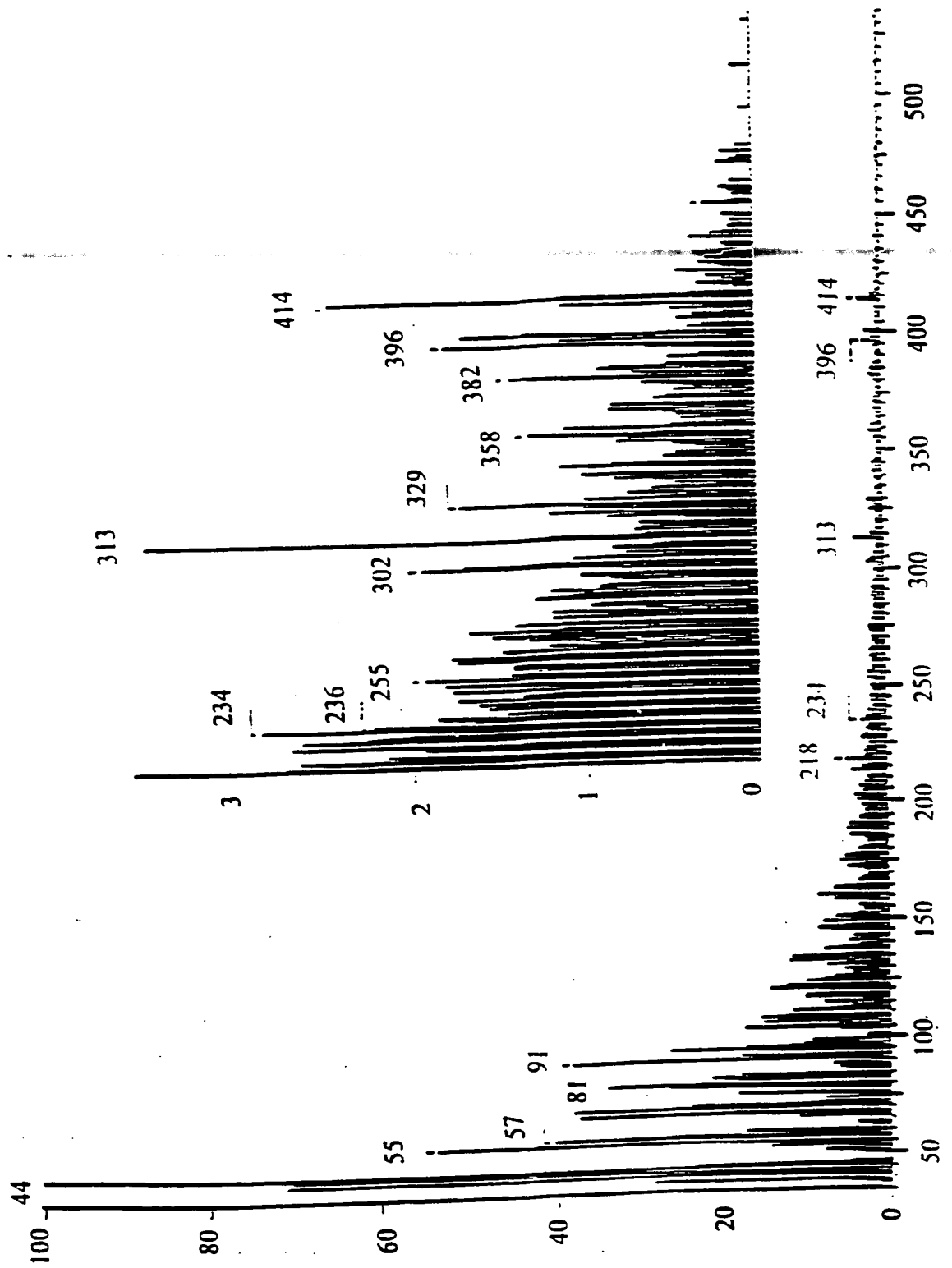
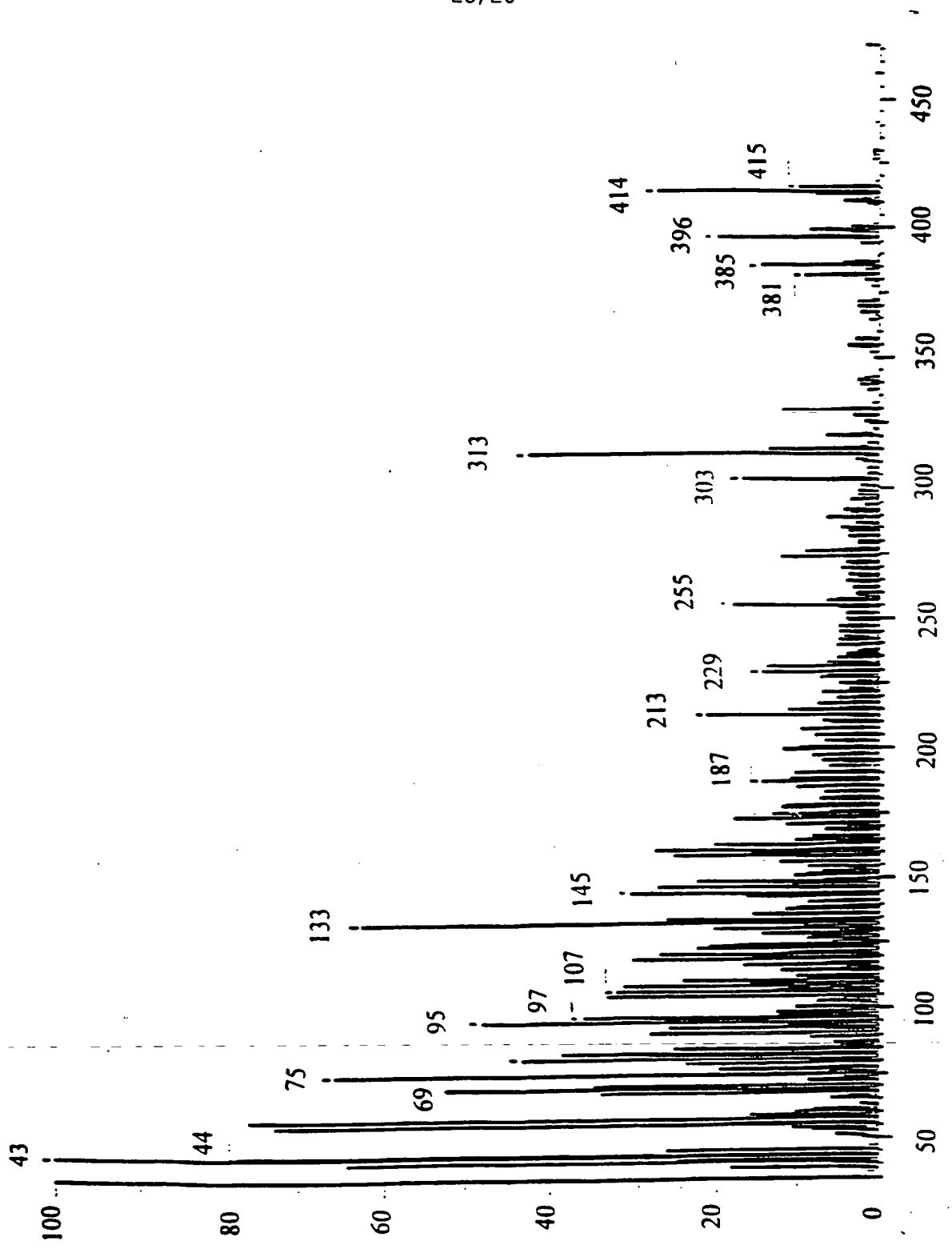


Figure 24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300
350
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450
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97
107
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145
187
213
229
255
303
313
381
385
396
414
415

Figure 25

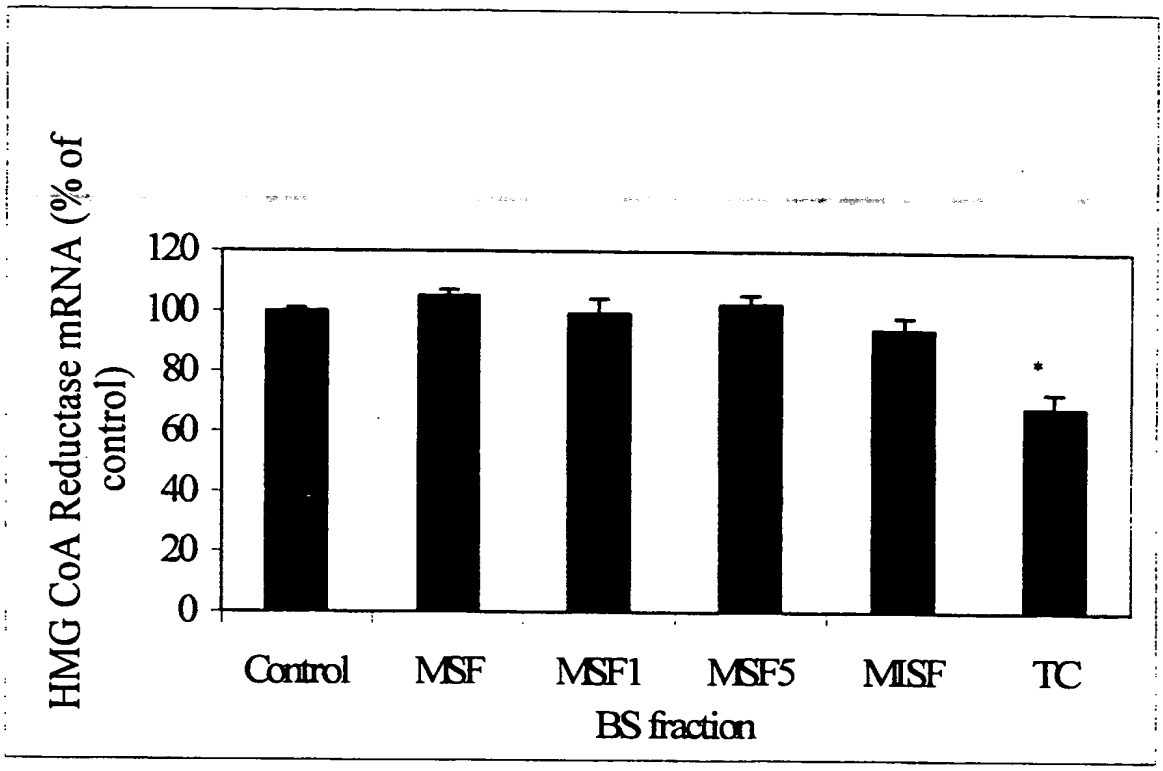

Figure 26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/12556

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A01N 65/00
US CL :424/195.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/195.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS
search terms: phytosterol, bamboo, bambusa, cholesterol

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,494,667 A (UCHIDA et al) 27 February 1996, see entire document.	1-37
A	US 5,466,453 A (UCHIDA et al) 14 November 1995, see entire document.	1-37

☐ Further documents are listed in the continuation of Box C.. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
19 AUGUST 1998	02 OCT 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer L. BLAINE LANKFORD Telephone No. (703) 308-0196

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